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13017

Prothrombin Level of Blood After the Intramuscular Injection of Sodium Citrate.*

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Shafiroff, Doubilet and Co Tui[1] have reported that the "prothrombin time" according to my method is decreased in dogs following the intramuscular injection of sodium citrate. Theoretically the "prothrombin time" can be altered by a change in the convertibility of prothrombin, by a further increase in thromboplastin, or by an actual alteration in the prothrombin level. A change in the convertibility of prothrombin remains a hypothetical conjecture which has never been satisfactorily demonstrated experimentally, although claimed by some investigators.[2] The decrease in "prothrombin time" may well be due to a liberation of thromboplastin from the platelets, but a change in the clotting time under these conditions can occur only if the thromboplastin employed in the test does not possess maximum potency. By using a thromboplastin having a high degree of activity, a minimum fixed clotting time is obtained, which cannot be further reduced by additional amounts of this activating factor. The most satisfactory preparation now known is rabbit brain dehydrated with acetone.[3] By means of this product, human plasma is clotted in 11 to 12½ seconds, and rabbit and dog plasma in 5½ to 6½ seconds.

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* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

[1] Shafiroff, B. G. P., Doubilet, H., and Co Tui, PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 136.

[2] Brinkhous, K. M., *Medicine*, 1940, **19**, 329.

Experimental. Dogs and rabbits were given sodium citrate intramuscularly and the prothrombin of the blood determined at regular intervals following the injection. Since the clotting time (prothrombin time) of undiluted plasma is very rapid, the test was also made on diluted plasma in which alterations in the prothrombin concentration cause relatively greater changes in the clotting time.

TABLE I.
Effect of Sodium Citrate (Intramuscularly)* on Prothrombin Level of the Blood.

		Clotting time (in seconds) of recalcified oxalated plasma with excess thromboplastin					
	Body wt kg	Before injection	30 min.	60 min.	90 min.	120 min.	
Dog A	12	6.5	6.5	6.5	6.5	6.5	
		12	12	11.5	11	11	Plasma diluted with 4 vol. saline sol.
" B	7	6.5	6.5	6.5	6.5	6	
		11.5	12.5	12.5	11.5	10	Plasma diluted as above
Rabbit A	2.9	5.5	5.5	5.5	5.5	5.5	
		11.5	11.5	12	12		" " " "
" B	2.1	5.5	5.5	5.5	5.5		
		11.5	11	11.5	11.5		" " " "

*Each animal received 0.5 g of sodium citrate (10% solution) per kg intramuscularly.

The prothrombin was determined by the author's method.[4] The thromboplastin used was made February 26, 1940, and preserved in an evacuated glass tube.

Results. The data recorded in Table I clearly demonstrate that the injection of sodium citrate did not alter the prothrombin level of the blood. The decrease in clotting time that is observed after the administration of sodium citrate as well as the apparent change of "prothrombin time" obtained by Shafiroff, *et al.*, is not due to a change in the prothrombin, but is brought about by some other factor. In view of the fact that a thromboplastin with high and constant activity can readily be prepared, there is no longer any valid justification for attempting quantitative studies of prothrombin with preparations of variable and uncertain potency.

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[3] Quick, A. J., *Science,* 1940, **92**, 113.

[4] Quick, A. J., *J. Am. Med. Assn.,* 1938, **110**, 1658.

Summary. The intramuscular injection of sodium citrate in dogs and rabbits does not change the prothrombin level of the blood.

13018

Determination of Pantothenic Acid in Normal Blood and Urine by Microbiological Technic.

MICHAEL J. PELCZAR, JR., AND J. R. PORTER.* (Introduced by William M. Hale.)

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In an earlier report from this laboratory[1] a technic was described for the assay of pantothenic acid based upon the growth response of the bacterium *Proteus morganii* to the vitamin. The application of this technic to the assay of pantothenic acid in natural substances, particularly blood and urine, is described in this communication.

Methods. The detailed procedure for the microbiological assay method employed has been given elsewhere.[2] Briefly, it consists of employing a pantothenate-free assay medium in which *Proteus morganii* will not grow. Addition of calcium pantothenate (Merck) in concentrations above 0.0001 μg per ml of medium permits growth of the test organism. Within limits the amount of growth is proportional to the pantothenate concentration. The increase in growth can be easily measured by employing a turbidimetric method. By plotting the turbidity values obtained against the concentration of pantothenate used, a "standard curve" is obtained.

To determine the pantothenic acid content of a specimen, an aqueous extract is prepared and autoclaved, and then a known quantity of this is incorporated in the assay medium. After inoculation with the test organism, the medium is incubated for 24 hours at 37°C and the turbidity is measured. Reference is then made to

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* We wish to thank Merck & Company, Inc., for generously supplying the synthetic calcium pantothenate employed in this study.

[1] Pelczar, M. J., Jr., and Porter, J. R., *J. Bact.*, 1941, **41**, 23.

[2] Pelczar, M. J., Jr., and Porter, J. R., *J. Biol. Chem.*, 1941, **139**, 111.

the "standard curve," and the pantothenate content of the extract is calculated.

Urine specimens were collected over a 24-hour period from 9 apparently normal people. These samples were collected at 4 different times during the experiment and precautions were taken to avoid bacterial contamination. To determine the pantothenate content of urine, a 1:100 dilution was made, and quantities of this dilution ranging between 0.1 ml-0.3 ml were incorporated in the assay medium.

Blood was obtained from normal individuals by pricking the finger tip and exactly 0.5 ml was added to 4.5 ml of distilled water. It was necessary to clarify this material prior to making additions to the medium. This was done by first heating the samples to boiling, then adding approximately 0.5 g of kielselguhr. After a few minutes the kieselguhr and adsorbed substances were removed by centrifugation. Following centrifugation the clear supernatant was removed, sterilized by autoclaving, and 0.4 ml added to the assay medium.

Results. Values obtained for the pantothenate content of normal blood and urine are presented in Tables I and II.

The results given in Table I for the pantothenate concentration of normal blood as determined by this investigation are not in agreement with the values obtained by Stanbery, Snell and Spies[3] who em-

TABLE I.
Pantothenic Acid Content of Normal Blood.

Source of specimen	Pantothenic acid concentration in blood (Specimens obtained on different days) I	II
	μg/ml	μg/ml
1	.054	.062
2	.070	.068
3	.070	.052
4	.047	.040
5	.099	.097
6	.081	.046
7	.032	.033
8	—	.030
9	.036	.054
10	.071	.058
11	.082	.080
12	.057	.060
13	.068	.084
14	.047	—
15	.031	.064
16	.048	.080
17	.049	—

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[3] Stanbery, S. R., Snell, E. E., and Spies, T. D., *J. Biol. Chem.*, 1940, **135**, 353.

TABLE II.
Pantothenic Acid Content of Normal Urine.

Source of Specimen	Pantothenic acid content of 24-hr specimens of urine collected on different days							
	I		II		III		IV	
	Vol.	Mg Pantothenic Acid	Vol.	Mg Pantothenic Acid	Vol.	Mg Pantothenic Acid	Vol.	Mg Pantothenic Acid
A	1950	4.39*	2050	4.27	1850	4.31	2250	3.26
B	1450	3.70	1425	3.98	—	—	1400	4.69
C	1900	5.05	—	—	—	—	—	—
D	2000	3.97	1900	2.60	1900	1.46	2650	3.71
E	1400	3.22	1150	2.76	1400	3.20	1400	3.36
F	1050	5.08	1200	4.86	1000	4.55	1250	4.44
G	1150	5.98	1350	4.59	1900	6.79	1450	4.13
H	800	2.74	1250	1.74	1000	2.55	1350	2.35
I	—	—	1000	4.30	700	2.31	1050	3.99

*Average results of repeated determinations.

ployed a different microbiological assay technic. They found that the concentration of this substance in normal whole blood ranged from 0.19-0.32 μg per ml and the average value obtained was 0.225 μg per ml. Our experiments indicate that a much lower level of this vitamin is present in normal blood. We have found that the content per ml of blood varies between 0.030-0.099 μg for the group of individuals studied and the average value, calculated from the results in Table I, is 0.059 μg per ml. We have also observed considerably more variation in specimens from the same individual, taken at different times, than was found by Stanbery, Snell and Spies.[3] The discrepancy between the results obtained in this investigation and those reported by Stanbery, Snell and Spies may be due to determination of the free pantothenic acid in the one instance and to the total pantothenate (free and combined) in the other.

The amount of pantothenic acid present in 24-hour specimens of urine obtained at 4 different times from 9 persons, varied from 1.46 mg to 6.79 mg. The calculated average was 3.81 mg. At present we are unaware of other similar data for comparison. Spies, Stanbery, Williams, Jukes and Babcock,[4] in an investigation on the relationship of pantothenic acid to nutrition in the human, measured the normal level of the vitamin in blood and urine as well as conditions influencing deviations from the normal. However, actual values for the individual determinations were not reported.

Evidence for the specificity of this assay procedure was obtained by recovery experiments. Known amounts of synthetic Ca-pantothenate were added to alkali-treated (pantothenate-free) urine and to other urine specimens whose pantothenic acid content had been previously determined. These materials were then assayed and the resulting values compared to the calculated amount of the vitamin which should be present. It will be seen that the recovery values presented in Tables III and IV are in very close agreement with the actual amounts calculated to be present. Similar experiments with blood showed that the adsorption procedure using kieselguhr did not lower the pantothenic acid content of the blood; the recovery of added Ca-pantothenate was quantitative. Additional evidence for the specificity of this technic is presented elsewhere.[2]

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[4] Spies, T. D., Stanbery, S. R., Williams, R. J., Jukes, T. H., and Babcock, S. H., *J. A. M. A.*, 1940, **115**, 523.

TABLE III.
Quantitative Recovery of Pantothenic Acid from Alkali Treated Urine.

Alkali treated urine specimens added to 10 ml of assay medium	µg pantothenic acid added to 10 ml of assay medium	Nephelometer reading	Pantothenic acid equivalent	% recovery
ml				
A—.05	.0	0	.0	—
	.002	23	.0019	95
	.004	42	.004	100
B—.05	.0	0	.0	—
	.002	22	.0018	90
	.004	42	.004	100
C—.05	.0	0	.0	—
	.002	24	.002	100
	.004	42	.004	100

TABLE IV.
Quantitative Recovery of Pantothenic Acid from Untreated Urine.

Additions made to 10 ml of assay medium		Total pantothenic acid content in assay medium		
Urine specimen (pantothenic acid content previously assayed) µg	Synthetic pantothenic acid, µg	Calculated pantothenic acid content, µg	Amt pantothenic acid as determined by assay, µg	% recovery
.0045	.001	.0055	.0051	92
	.002	.0065	.0061	94
.0033	.001	.0043	.0044	102
	.002	.0053	.0057	107
.0022	.001	.0032	.0030	94
	.002	.0042	.0043	102

Summary. A microbiological technic, employing *Proteus morganii* as the test organism for the assay of pantothenic acid, has been applied to evaluating the level of this vitamin in blood and urine.

It was found that the pantothenate content of blood from 17 normal persons ranged between 0.030 and 0.099 μg per ml of blood and averaged 0.059 μg per ml. The pantothenate content of 24-hour urine specimens from 9 persons ranged from 1.46 mg to 6.79 mg and averaged 3.81 mg.

13019 P

Experimental Alimentary Azotemia: Comparative Effects of Red Cells and Plasma.

C. FRANK CHUNN AND HENRY N. HARKINS.

From the Division of General Surgery, Henry Ford Hospital, Detroit.

In a previous paper,[1] the authors showed in experimental animals that the ingestion of whole blood produces a marked rise in the urea nitrogen content of the recipient's blood. This observation agreed with that of Schiff, *et al.*,[2] Kaump and Parsons,[3] and Yuile and Hawkins[4] and indicated that the azotemia occurring in clinical cases of bleeding peptic ulcer might also be caused by blood absorption.

In an attempt to find what element in the blood caused the rise, 5 dogs were given beef plasma by stomach tube in the first series of experiments and in the second series of experiments 5 dogs were given beef cells in the same manner. In all 10 experiments blood samples were taken every day at 2 a.m., 8 a.m., 12 noon, 4 p.m., and 8 p.m., one day before giving the substance to be tested and for at least 2 days thereafter. In all instances the dogs given cells showed a much more marked elevation in blood urea nitrogen than did the plasma fed dogs. A typical curve is shown in Chart 1.

Effect of hemoglobin. Administration of a pure hemoglobin solution to 2 dogs produced a rise in blood urea nitrogen comparable to that produced by cells alone.

Effect of iron. To rule out the action of the iron in the hemo-

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[1] Chunn, C. F., and Harkins, H. N., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 569.

[2] Schiff, L., Stevens, R. J., Goodman, S., Garber, E., and Lublin, A., *Am. J. Dig. Dis.*, 1939, **6**, 597.

[3] Kaump, D. H., and Parsons, J. C., *Am. J. Dig. Dis.*, 1940, **7**, 191.

[4] Yuile, C. L., and Hawkins, W. B., *Am. J. Med. Sci.*, 1941, **201**, 162.

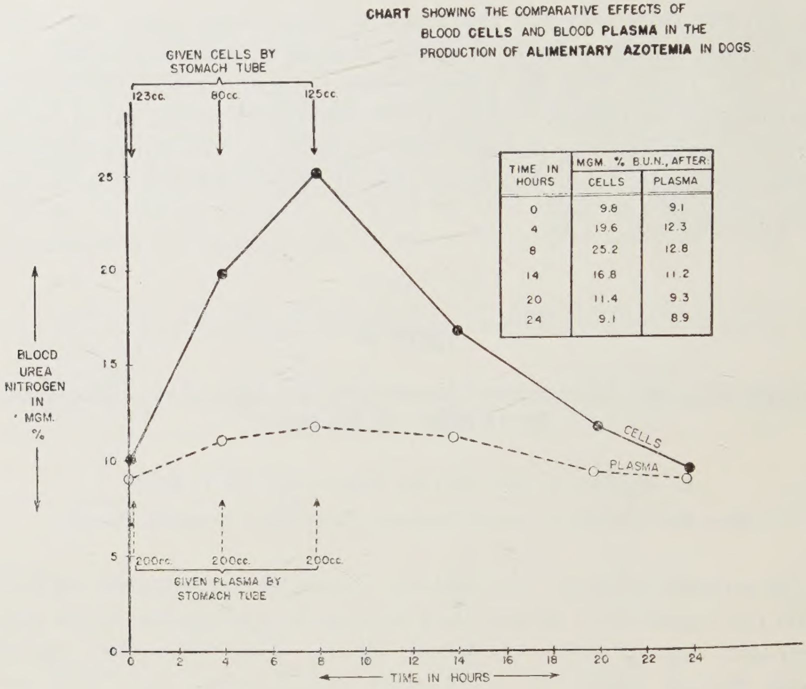
CHART SHOWING THE COMPARATIVE EFFECTS OF BLOOD **CELLS** AND BLOOD **PLASMA** IN THE PRODUCTION OF **ALIMENTARY AZOTEMIA** IN DOGS

TIME IN HOURS	MGM. % B.U.N., AFTER:	
	CELLS	PLASMA
0	9.8	9.1
4	19.6	12.3
8	25.2	12.8
14	16.8	11.2
20	11.4	9.3
24	9.1	8.9

FIG. 1.

globin, 2 dogs were given 1 g of iron each by stomach tube in the form of 10% iron ammonium citrate (one gram of iron is the amount normally present in 1000 cc of blood). No azotemia resulted.

Effect of protein. Two dogs were given a single large protein meal consisting of casein and ground beef, the first dog receiving 83 g of protein and the second 175 g. A marked rise in blood urea nitrogen occurred.

Effect of urea. Experiments were done to confirm the common observation that urea alone will produce a blood elevation when given by mouth. However, the urea content of the beef cells used in our experiments listed above was not appreciably greater than the urea content of beef plasma. Therefore, the urea inherent in the cells could not have been the important factor in causing the alimentary azotemia.

Conclusions. Alimentary azotemia results from blood absorption from the intestinal tract. The important blood elements in this regard are the red cells and particularly their contained hemoglobin. The protein fraction rather than the iron content is the active portion of the hemoglobin.

13020 P

Relative Effect of Blood Given by Jejunostomy and Ileostomy.

C. Frank Chunn, Henry N. Harkins and Robert T. Boals.
From the Division of General Surgery, Henry Ford Hospital, Detroit.

Previous experiments[1, 2] have indicated that blood or red blood cells when introduced by stomach tube into normal dogs will produce a marked rise in blood urea nitrogen. These experiments also indicated that the red cells and particularly their contained hemoglobin seemed to be the active factor in producing this alimentary azotemia rather than the plasma. Finally, since marked rises in blood urea nitrogen were also obtained following ingestion of protein, it seems as though the rise is probably due to and to a large extent proportional to the absorption of protein from the gastrointestinal tract.

All of the above conclusions were based on the results of introducing blood by tube directly into the stomachs of experimental animals, while the following experiments were performed by introducing blood or red blood cells into the jejunum and ileum of dogs.

Method. Ten experiments were performed on 4 dogs. Two of the dogs had a jejunostomy of the Maydl type 2 feet below the ligament of Treitz and the other 2 dogs had an ileostomy of the Mann-Bollman type 20 cm proximal to the ileocecal value. The blood or red blood cells was introduced by tube into these enterostomy openings. In 2 experiments whole beef blood was used, in 5 beef red blood cells and in 3 human red cells were used.

Blood urea nitrogen determinations, using essentially the method of Van Slyke and Cullen[3] were made every 4 to 6 hours on each dog, from one to 3 days following the introduction of whole blood or of red blood cells by enterostomy into the respective levels of the small intestine. As in the previous experiments on oral administration, the dogs were given their usual amount of food divided into 4 small meals daily. Water was given freely in the usual amounts.

Results. Whole blood administration. Following the administration of whole beef blood into the jejunum, there was a marked rise in blood urea nitrogen content within 3 hours from the intro-

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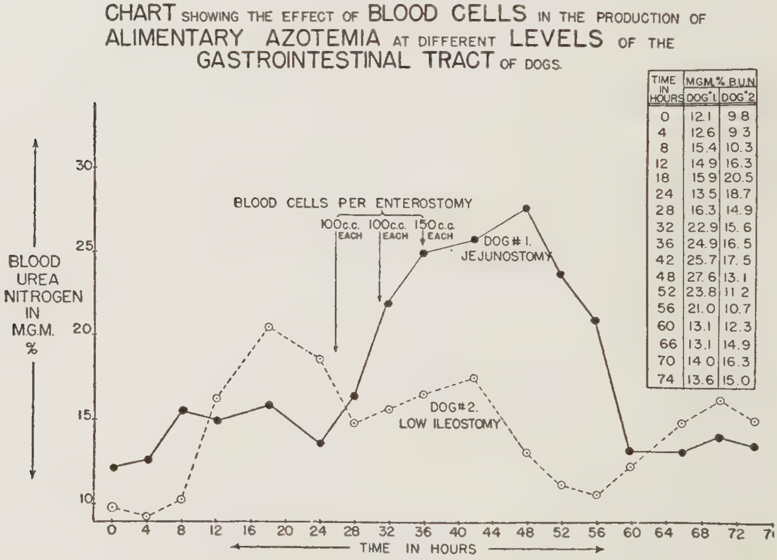
[1] Chunn, C. F., and Harkins, H. N., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 569.

[2] Chunn, C. F., and Harkins, H. N., *Surgery,* 1941, **9**, 695.

[3] Van Slyke, D. D., and Cullen, G. E., in Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry,* Williams and Wilkins Company, Baltimore, 1932, **2**, 547.

duction of the first dose of blood with a return to the normal level 24 hours after the introduction of the last dose of blood. In marked contrast to this course, was the result of giving as much as 750 cc blood into the lower ileum where no elevation of blood urea nitrogen occurred.

Results. Red blood cells administration. In all cases except one, the administration of red blood cells produced the same results as the use of whole blood. The one exception was a dog in which giving of human red blood cells by low ileostomy was accompanied by an increase in blood urea nitrogen of from 14 to 24 mg %. It was then found that this dog had received a single large feeding of meat just before this rise occurred which may have been a causative factor. A typical set of experiments is shown on Chart 1, illustrating the contrast between the results produced by red blood cells administration into a jejunostomy and into a low ileostomy.



TIME IN HOURS	MGM. % B.U.N. DOG # 1	DOG # 2
0	12.1	9.8
4	12.6	9.3
8	15.4	10.3
12	14.9	16.3
18	15.9	20.5
24	13.5	18.7
28	16.3	14.9
32	22.9	15.6
36	24.9	16.5
42	25.7	17.5
48	27.6	13.1
52	23.8	11.2
56	21.0	10.7
60	13.1	12.3
66	13.1	14.9
70	14.0	16.3
74	13.6	15.0

Fig. 1.

Results. Whole blood intraperitoneally. Three normal dogs were given 150 cc, 200 cc, and 230 cc respectively of citrated whole blood intraperitoneally, using aseptic technic. No elevation of blood urea nitrogen occurred in any of the 3 experiments.

Comment. Correlation of these results with those of experiments where blood was administered by mouth would indicate that alimentary azotemia only results when blood is placed in the upper half of the intestinal tract. This may be because of a necessary digestion in or absorption from this portion of the gut. Our results cannot give a conclusive answer as to which of these factors is more important. Administration of a 5% urea solution produced as marked a rise in blood urea nitrogen when introduced into the stoma of one of the low ileostomy dogs as when introduced into one of the jejunostomy dogs. This would indicate the importance of the element of digestion but is not conclusive because urea is such a readily diffusible substance.

Conclusions. Alimentary azotemia in the dog occurs after the intragastric or intrajejunal administration of whole blood or red blood cells. On the other hand, similar amounts introduced low into the ileum or into the peritoneal cavity produced practically no rise in blood urea nitrogen. Alimentary azotemia results, then, only from the presence of blood in the upper intestinal tract.

13021

Detection of Minute Amounts of Serum Antibody by Agglutination of Antigen-Coated Bacterial Cells.

EUGENE C. ROBERTS AND LLOYD R. JONES.

From the Department of Bacteriology, St. Louis University School of Medicine.

The authors recently described a local serum reaction[1] occurring upon the ear of a rabbit (at the site of a prior injection of serum antigen) when a very small quantity of homologous antiserum was injected intravenously elsewhere in the body. The amount of antiserum injected was so small that it was not possible to detect antibody in the animal's serum by the use of the conventional method of testing, *i. e.,* the precipitation test employing the antigen dilution method. In order to determine such minute amounts of circulating antibody in the blood of injected animals it became necessary to devise a technic which would be both accurate and extremely sensitive. This was accomplished by developing a method which involves coating the cells of a suspension of *Serratia marcesens* with serum antigen and observing the agglutination of such cells upon addition of the animal serum being tested. That bacterial cells can be coated with serum antigen and subsequently agglutinated with an immune serum (homologous to the serum antigen) was reported by Jones.[2]

---

[1] Jones, L. R., and Roberts, E. C., *J. Immunol.*, 1941, **40**, 107.

[2] Jones, F. S., *J. Exp. Med.*, 1927, **46**, 303.

However, to our knowledge, no practical application of this type of antigen-antibody reaction has been made heretofore.

It became evident in preliminary experiments, that agar grown cells (washed twice with saline solution, suspended in distilled water and killed by flowing steam) could be satisfactorily coated by suspending them in horse or beef serum (antigen) and incubating the mixture at 37°C for not less than 9 hours. With such sera in order to achieve satisfactory coating it was necessary that the quantitative ratio of serum to cells be of the order of 5 to 1, as based upon the nitrogen contents of these materials. Electrolyte content of serum does not interfere with satisfactory adsorption of antigen upon the cells. This is a particular advantage of using bacteria rather than collodion particles as the particulate agents for antigen adsorption, as Cannon[3] has reported that in order to secure satisfactory coating upon collodion particles, the antigen solution should be dialyzed as free as possible from any dissolved salts.

The suspension, above referred to, is centrifuged to remove the antigen coated cells, these in turn are washed twice with saline solution and finally diluted in saline to the desired opacity (equivalent to a reference standard containing 0.25 mg of ground Pyrex glass per ml). By centrifuging gently (200 r.p.m.) for 5 minutes, the larger clumps or aggregates of bacteria are removed. Whereupon 0.5 ml of the supernatant suspension of coated bacterial cells is added to each of a series of small tubes to make the agglutination test. One ml quantities of serial dilutions of antiserum (fresh or inactivated) are added to the tubes. All tubes, including suitable controls are then incubated for 2 hours at 40°C, following which they may be read at once or placed in the refrigerator over night before making the final readings. Agglutination is of a fine granular type and corresponds to that which is ordinarily observed with 'O' type suspensions of bacteria. Employing this technic we have not observed agglutination with any of the sera of a group of normal rabbits.

In Table I is recorded the antibody titers, as determined by the conventional precipitation test employing the antigen dilution method and this newly devised technic which involves bacterial agglutination (B.A. method) of antigen coated cells at various time intervals after the intravenous injection of a rabbit with a single dose of rabbit anti-horse serum.

Table II similarly indicates the titers observed (2 hours post-injection) in rabbits receiving varying quantities of the same antiserum.

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[3] Cannon, P. R., and Marshall, C. E., *J. Immunol.*, 1940, **38**, 265.

TABLE I.

Antibody Titers in the Serum of a Rabbit Passively Immunized with a Single Dose of Rabbit Anti-Horse Serum.*

Time interval after antiserum administration	Precipitation test	B.A. method
2 hr	+ undil.	1:512
24 ”	+ ”	1:512
4 days	—	1:128
7 ”	—	1:32
9 ”	—	1:8
11 ”	—	1:16
12 ”	—	1:16

*Intravenous injection of 1 ml per kg. (Precipitin titer 1:60,000).

TABLE II.

Antibody Titers in Serum of Rabbits Two Hours After Passive Immunization with a Single Dose of Rabbit Anti-Horse Serum.

Antiserum amt inj. per kg, ml	Precipitation test	B.A. method
1.0	+ undil.	1:512
0.5	—	1:64
0.25	—	1:16
0.125	—	1:2

These tables indicate that the B.A. method possesses greater sensitivity in the detection of serum antibody than the conventional precipitation test. This greater sensitivity is again depicted in Table III, wherein the 2 tests were employed to detect the presence of antibody in an animal actively immunized with a single, small dose of horse serum.

TABLE III.

Antibody Titers in the Serum of a Rabbit Actively Immunized with a Single Dose of Horse Serum.*

	Precipitation test	B.A. test
Before antigen inj.	—	—
24 hr after ”	—	1:4
2 days ” ”	—	1:64
3 ” ” ”	—	1:256
4 ” ” ”	+ undil.	1:512
5 ” ” ”	—	1:64
6 ” ” ”	—	1:64

*Subcutaneous injection of 0.15 ml per kg.

It is significant that antibody was observed not only much earlier with the B.A. method, but in addition antibody could be detected 24 hours after antigen administration. The early appearance of antibody, detectable by this sensitive technic, has been confirmed in other similarly injected animals.

During the course of this study, the blood sera of a number of human beings who were receiving relatively large amounts of an antigenic foreign protein, were made available to us at intervals during the course of their therapeutic programs.* These sera were tested for specific antibody content by the conventional precipitation test and by the bacterial agglutination (B.A.) technic herein described. A comparison of the results obtained by the 2 methods of antibody determination indicates that the B.A. method when also applied to the serum of man serves to detect antibody earlier and that significant titers persist longer than is observed with the conventional precipitation test.

It seems likely, therefore, that the B.A. method of antibody detection possesses certain advantages over the conventional precipitin test as made by the antigen dilution method. While the B.A. method may not be more sensitive than the collodion particle agglutinative method as described by Freund,[4] by Cannon and Marshall,[3] and others, we have found, however, that it is more convenient and that the bacteria employed exhibit greater suspension stability than collodion particles.

Since the B.A. method is capable of detecting extremely small quantities of antibody in serum it is conceivable that it may be advantageously applied to various immunological problems, *e. g.*, allergic states, virus diseases and other conditions wherein very small amounts of circulating antibody may be involved in the mechanism of the disorder. Our current studies involving the use of this newly devised technic in a virus disease (encephalitis of the St. Louis type) will be reported subsequently, wherein it will be shown that the sera of convalescent patients agglutinate bacterial cells treated with mouse brain virus antigen.

13022 P

Paradoxical Blood Concentrating Effect of Intravenous Four Normal Plasma Injections.

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From the Division of General Surgery, Henry Ford Hospital, Detroit.

If plasma is injected intravenously, one would expect the blood to become diluted. If concentrated plasma is similarly injected, because of osmotic attraction of fluid from the tissues, one would expect the blood to become even more diluted than following the intravenous injection of normal plasma.

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* These sera were supplied by Dr. R. O. Muether and the precipitation tests were made by Miss Ruth Kettler.

[4] Freund, J., *Am. Rev. Tbc.*, 1925, **12**, 124.

These theoretical expectations outlined in the previous paragraph were not supported by our experiments, which gave exactly the opposite results. Experiments were performed on healthy dogs under nembutal anesthesia. Large doses of reconstituted 4 normal dried dog or human plasma (prepared by the method of Hartman[1]) were rapidly injected intravenously and numerous blood samples were taken from an ear artery for hematocrit and hemoglobin estimations. An immediate paradoxical blood concentration occurred which lasted for about 30 minutes and was not followed by any secondary blood dilution.

Similar experiments were performed using 4 x normal glucose solution (20%). The results were the exact opposite of the plasma injections and revealed a marked immediate dilution as would be expected. Typical curves are shown in Fig. 1.

Calculations can be made assuming the vascular tree of the recipient dogs to be a closed system, thus:

A. Glucose injection	*B. Concentrated plasma injection*
Dog wt—12.0 kg	Dog wt—9.7 kg
Calc. blood vol.—920 cc ($1/13$ body wt)	Calc. blood vol.—745 cc ($1/13$ body wt)
Control hematocrit—44	Control hematocrit—38
Calc. control erythron—405 cc.	Calc. control erythron—285 cc
Calc. control plasma vol.—515 cc	Calc. control plasma vol.—460 cc
Add 120 cc 20% glucose	Add 100 cc 4 x normal plasma
Final erythron still—405 cc	Final erythron still—285 cc
Final plasma vol. now—635 cc	Final plasma vol. now—560 cc
Final calc. hematocrit—39	Final calc. hematocrit—34
(Final observed hematocrit—33)	(Final observed hematocrit—45)

From these calculations it is seen that glucose produces more of a blood dilution than would be expected (decrease of 11 in the hematocrit instead of 5), while plasma causes the reverse effect (increase of 7 instead of the calculated decrease of 4 in the hematocrit). The excess dilution produced by glucose may be caused by an immediate inflow of tissue fluids into the blood stream, while there is no evidence for such a response to concentrated plasma injections.

A search of the literature revealed only two articles bearing on our results. Brennan[2] noted an increase in the red blood count in acutely anemic neurosurgical patients following the injection of half-normal plasma. He also noted a decrease in the mean corpuscular volume, which did not occur in our experiments, since the alterations in the hemoglobin paralleled those of the hematocrit.

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[1] Hartman, F. W., and Hartman, F. W., Jr., *J. A. M. A.*, 1940, **115**, 1989.

[2] Brennan, H. J., *Brit. M. J.*, 1940, **1**, 1047.

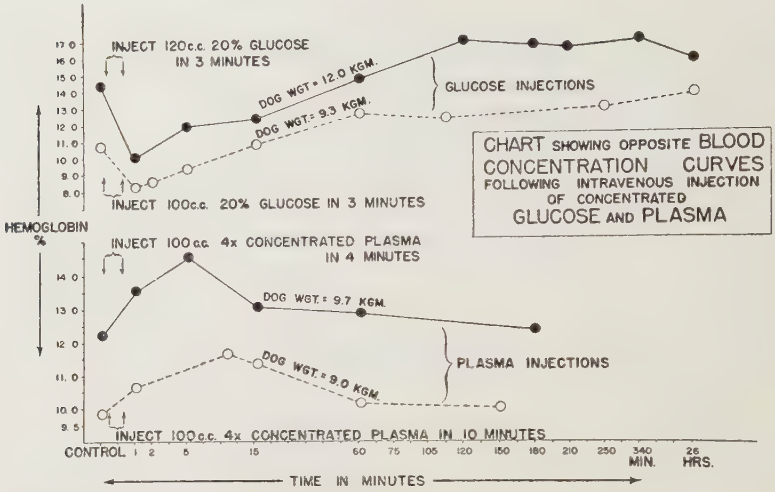

Fig. 1.

Keeley, Gibson and Pijoan[3] found that following burns, there was an increase in circulating erythrocytes in normal animals, but a decrease in splenectomized animals. This latter paper suggested the possibility that in our experiments the blood concentration resulted from splenic contractions. One splenectomized dog so far studied showed a blood dilution following 4 normal plasma injections, rather than the usual paradoxical blood concentration.

Conclusions. (1) The intravenous injection of concentrated glucose solution causes an immediate blood dilution. This dilution is greater than would be expected if the vascular tree were a closed system. (2) The intravenous injection of concentrated dog and human plasma under similar conditions not only causes no dilution, but a paradoxical blood concentration.

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[3] Keeley, J. L., Gibson, J. G., 2nd, and Pijoan, M., *Surgery*, 1939, **5**, 872.

13023

Effect of Feeding Wheat Germ Oil on Production of Liver Cancer by Butter-Yellow.

KANEMATSU SUGIURA.

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Yoshida[1] experimentally produced liver cancer in rats by feeding o-aminoazotoluene, and Kinosita,[2] using dimethylaminoazobenzene, commercially known as butter-yellow, produced liver cancer in a much shorter time. In both cases, rice was used as the basal food for the animals. On the other hand, Fischer-Wasels[3] reported that rats receiving o-aminoazotoluene and bread developed only liver cirrhosis.

Recently Kinosita[4] and Okada[5] showed that the production of liver cancer by dimethylaminoazobenzene is definitely reduced when wheat bread was used as basal diet instead of rice. Vassiliadis[6] found that rats receiving o-aminoazotoluene and a diet of wheat flour showed no tumor at the end of a year. Whereas, if the diet consisted solely of rice and dye substance, a large number of adenocarcinoma of the liver appeared. The failure of o-aminoazotoluene to produce liver cancer in rats maintained on the diet of wheat was also shown by Ando.[7] These experiments demonstrate that a certain substance of wheat is antagonizing the carcinogenic action of dimethylaminoazobenzene or of o-aminoazotoluene in the body.

The object of the present study is to find whether or not wheat germ oil has any inhibiting effect upon the production of liver cancer in rats induced by the oral administration of butter-yellow. It was shown in a recent communication[8] that the ether extracts of rice-bran and of brewer's yeast contain a substance which is inhibitory to the development of liver cancer in rats by butter-yellow feeding.

Experimental. In the present series of experiments two samples of wheat germ oil were used. One was prepared in the same manner as that of rice-bran oil, *i. e.,* the wheat germ was continuously extracted in Soxhlet extractors with ether for 24 to 48 hours at 50°C. One thousand grams of wheat germ yielded about 70 cc of a yellowish brown oily emulsion. Another sample of wheat germ oil was kindly prepared by Dr. C. Hoffman of Ward Baking Co., N. Y. One hundred cc of the oil represented 900 g of wheat germ. It contained 0.5 gamma of riboflavin (vitamin B_2) per gram of the material.

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[1] Yoshida, T., *Proc. Imp. Acad. Japan*, 1932, **8**, 464.

[2] Kinosita, R., *Trans. Soc. path. jap.*, 1937, **27**, 665.

[3] Fischer-Wasels, B., *Zbt. allg. Path. Anat.*, 1936, **66**, 359.

[4] Kinosita, R., *Gann*, 1939, **33**, 225.

[5] Okada, D., *Osaka Igaku Zasshi*, 1940, **39**, 485.

[6] Vassiliadis, H. C., *Am. J. Cancer*, 1940, **39**, 377.

[7] Ando, T., *Gann*, 1940, **34**, 356.

[8] Sugiura, K., and Rhoads, C. P., *Cancer Research*, 1941, **1**, 3.

A group of 10 rats was maintained on the mixture of 95% unpolished rice and 5% of the wheat germ oil prepared here. A second group of 10 rats was maintained on the mixture of 95% unpolished rice and 5% wheat germ oil prepared by the Ward Baking Co. A third group of 20 rats was kept on unpolished rice without wheat germ oil. All animals received butter-yellow (p-dimethylaminoazobenzene) in the proportion of 20 cc of 3% solution in olive oil per 1000 g of food. Each of the 3 groups of rats received a small amount of fresh carrot daily. Each rat consumed approximately 5 to 7 g of food daily.

After 150 days the experiments were discontinued; all rats then living were sacrificed and examined. The results of the experiments are presented in Tables I, II, and III. In all cases the gross diagnoses were confirmed by microscopic examination. Rats used in this study were of the Sherman strain. Rats that died before the period of 60 days are not included in the tables.

TABLE I.
Effect of Feeding Wheat Germ Oil on Production of Liver Cancer by Butter-Yellow.

Rat No.	Sex	No. of days fed	Body weight (g)			Liver findings*
			Initial	Maximum	Final	
1	♀	68	112	112	95	±
2	”	84	122	122	87	±
3	”	107	130	130	68	+
4	♂	132	112	132	127	+
5	”	150	135	143	136	+
6	”	150	122	122	75	+
7	♀	150	126	126	100	+
8	♂	150	122	143	130	+
9	”	150	120	129	113	++

* — indicates smooth, practically normal liver; ± indicates nodular cirrhosis with adenomatous hyperplasia; + indicates distinct areas of cholangioma or hepatoma or both; ++ indicate extensive liver cancer with or without metastasis.

The data in Tables I and II show clearly that daily ingestion of wheat germ oil, rich in vitamin E but poor in vitamin B_2 had no protective action upon the production of liver cancer. Of the 16

TABLE II.
Effect of Feeding Wheat Germ Oil (Ward Baking Co.) on Production of Liver Cancer by Butter-Yellow.

Rat No.	Sex	No. of days fed	Body weight (g)			Liver findings*
			Initial	Maximum	Final	
1	♀	70	127	127	80	±
2	"	101	130	130	70	+
3	♂	106	134	150	86	+
4	"	114	135	135	70	+
5	♀	114	128	128	77	+
6	♂	141	140	140	109	++
7	♀	150	141	141	128	—
8	"	150	135	139	125	+
9	♂	150	138	138	120	++
10	"	150	135	148	136	++

*See Table I for explanation of symbols.

TABLE III.
Effect of Unpolished Rice Alone on Production of Liver Cancer by Butter-Yellow.

Rat No.	Sex	No. of days fed	Body weight (g)			Liver findings*
			Initial	Maximum	Final	
1	♀	66	125	125	75	—
2	"	66	125	134	110	±
3	♂	68	119	119	88	±
4	♀	68	138	138	97	±
5	♂	74	127	127	70	+
6	♀	85	124	124	107	+
7	♂	103	116	118	70	+
8	♀	105	127	129	100	+
9	"	107	130	130	68	+
10	"	109	116	116	69	+
11	♂	124	130	130	70	+
12	♀	143	118	118	60	+
13	♂	150	144	146	145	+
14	"	150	131	135	100	+
15	♀	150	132	132	83	+
16	♂	150	121	121	105	++
17	♀	150	120	120	109	++

*See Table I for explanation of symbols.

rats which died or were sacrificed between 100 and 150 days after the beginning of the experiment, microscopic examination revealed that 15 animals (94%) had liver cancer, both cholangioma and hepatoma.

The author wishes to express his appreciation to Dr. C. P. Rhoads for his interest and valuable advice.

13024

Inhibitory Effect of Sulfamido Compounds upon Development and Growth of Phage-Resistant Bacteria.

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Zaytzeff-Jern and Meleney[1] reported that, in contrast to many disinfectants, sulfanilamide and sulfapyridine do not interfere with the lytic action of bacteriophage, but may even enhance its lytic activity. The observations of these authors on the synergistic action of sulfamido compounds and staphylococcus bacteriophage could be confirmed. Furthermore, it was found that these chemotherapeutic substances inhibit or delay the development of phage-resistant staphylococcus. It was decided, therefore, to study the effect of sulfamido compounds upon development and growth of phage-resistant bacteria. The results of this investigation are presented in the following communication.

The experiments were carried out with staphylococcus, *S. dysenteriæ* (Shiga), and *E. coli* bacteriophage and the respective susceptible strains. As culture medium, brain heart infusion broth (Difco), containing infusion from calf brains and beef heart, 1% proteose-peptone, 0.2% dextrose, 0.5% sodium chloride, and 0.25% disodium phosphate, was used in all experiments.

Sulfanilamide (p-aminobenzenesulfonamide, prontylin, repurified for injection, Winthrop); sulfapyridine (2-sulfanilylaminopyridine, Merck); sulfathiazol (2-sulfanilamidothiazol, Squibb); sulfadiazine (2-sulfanilamido-pyrimidine, Lederle); and sulfanilylguanidine monohydrate were dissolved in appropriate amounts in broth by heating in a water bath. The material was then sterilized by autoclaving at 15 lb pressure for 12 minutes.

In the first experiment the effect of sulfathiazol upon the development and growth of phage-resistant staphylococci in infusion broth containing staphylococcus bacteriophage was studied. The bacteriophage was diluted in broth and in broth containing 125 mg % of sulfathiazol (volume 20 cc) ranging from 10^{-2} to 10^{-10}. Broth and sulfathiazol-broth were used as controls. Then all flasks were seeded with 0.2 cc of a 1:10,000 diluted 24 hours infusion broth culture of *Staphylococcus aureus hemolyticus*. The strain (7011)

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[1] Zaytzeff-Jern, H., and Meleney, F. L., *J. Lab. Clin. Med.*, 1939, **24**, 1017.

TABLE I.

Action of Sulfathiazol (125 mg%) upon Development of Phage-Resistant Staphylococci in Infusion Broth Containing Bacteriophage.

Dilution of phage in broth	Time of Incubation												
	Hours				Days								
	18	48	72	96	6	8	9	12	14	15	17	21	23
10-2	—	—	—	—	—	—	—	—	++++	++++	++++	++++	++++
10-4	—	—	+	++	++++	++++	++++	++++	++++	++++	++++	++++	++++
10-6	—	+++	+++	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++
10-8	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
10-10	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
0	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Dilution of phage in sulfathiazol broth													
10-2	—	—	—	—	—	—	—	—	—	—	—	—	+
10-4	—	—	—	—	—	—	—	—	—	—	—	++	+++
10-6	—	—	—	—	—	—	—	—	—	—	—	—	—
10-8	—	—	—	—	—	—	—	—	—	—	—	—	—
10-10	++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
0	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++

— = no visible growth. + to ++++ = various degrees of visible growth.

TABLE II.

Effect Sulfanilyl-Guanidine on Development of Phage-Resistant Shiga Dysentery Bacilli in Infusion Broth Containing Bacteriophage.

		A—Broth containing sulfanilyl-guanidine in concentrations of					B—Bacteriophage (10-6) in broth containing sulfanilyl-guanidine in concentrations of				
	Time of incubation	a 150	b 15	c 1.5	d 0.15	e 0	a 150	b 15	c 1.5	d 0.15	e 0
		mg%									
1.	18 hr	+++	++++	++++	++++	++++	—	—	—	—	—
2.	48 ”	+++	++++	++++	++++	++++	—	—	—	—	+++
3.	72 ”	+++	++++	++++	++++	++++	+	—	—	—	+++
4.	96 ”	+++	++++	++++	++++	++++	—	—	+++	++	+++
5.	6 days	+++	++++	++++	++++	++++	++	—	+++	++	+++
6.	7 ”	+++	++++	++++	++++	++++	++	—	+++	++	+++
7.	8 ”	+++	++++	++++	++++	++++	++	—	+++	+++	+++
8.	10 ”	+++	++++	++++	++++	++++	++	+	+++	+++	+++
9.	14 ”	+++	++++	++++	++++	++++	++	++	+++	+++	+++

— = no visible growth. + to ++++ = various degrees of visible growth.

was freshly isolated from a child with staphylococcal empyema. The flasks were incubated at 37°C for 23 days and the resulting growth and clearing were noted at various intervals. The results of this experiment are presented in Table I.

Table I shows that in the presence of 125 mg % of sulfathiazol bacteriophage is effective in a higher dilution (10^{-8}) than bacteriophage alone (10^{-6}). Although this difference is not great, the same results were obtained in repeated experiments. The table, furthermore, reveals that the development of phage-resistant staphylococci is markedly delayed or even inhibited in the presence of sulfathiazol. Essentially the same results were obtained in several experiments with sulfathiazol, sulfapyridine (150 mg %) and sulfanilamide (500 mg %).

The experiments on the inhibitory effect of sulfathiazol on the development of phage-resistant staphylococci in broth containing bacteriophage are of particular interest, since at the present time this chemotherapeutic agent is the drug of choice in the treatment of severe staphylococcal infections. There is evidence available that bacteriophage does not cause lysis of the organisms *in vivo,* but rather promotes phagocytosis (MacNeal, McRae and Colmers[2]). This suggests experiments on the effect of sulfamido compounds on the phagocytosis of staphylococci in the presence of bacteriophage.

In the next series of experiments the effect of various sulfamido compounds upon the development of phage-resistant bacilli was investigated. *S. dysenteriæ* (Shiga) and *E. coli* and the respective bacteriophages were employed. The results of these experiments were not as striking as those obtained with staphylococci. The development of phage-resistant bacilli was usually not entirely prevented. However, in the majority of experiments sulfanilamide (1000 mg %), sulfapyridine (100-150 mg %), sulfadiazine (150 mg %) and sulfanilyl-guanidine delayed and partly inhibited the secondary growth of both *E. coli* and *S. dysenteriæ* (Shiga). A typical experiment ensues: Shiga bacteriophage was diluted one million fold (volume 5.0 cc) in broth containing sulfanilyl-guanidine in concentrations of 150 mg %, 15 mg %, 1.5 mg %, and 0.15 mg %. As controls broth containing sulfanilyl-guanidine in the same concentrations, but not bacteriophage, was used. The tubes were seeded with 0.2 cc of 1:100 diluted 24 hours brain heart infusion broth culture of Shiga dysentery bacillus. The tubes were incubated at 37°C for 14 days. Growth was noted at various intervals. The results of this experiment are presented in Table II. It is noteworthy that both phage-resistant *E. coli* and *S. dysenteriæ* on subculture proved to be susceptible to the bacteriostatic action of sulfanilamide and sulfapyridine. It remains to be seen whether *in vivo* administration of both bacteriophage and sulfanilyl-guanidine —a drug of promise in the treatment of localized bacillary infections of the intestinal tract (Marshall[3])—or other sulfanilamide derivatives may be more efficacious than treatment with either agent alone.

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[2] MacNeal, W. J., McRae, M. A., and Colmers, R. A., *J. Infect. Dis.*, 1938, **63**, 25.

Summary. (1) Sulfanilamide, sulfapyridine and sulfathiazol either completely inhibit or definitely delay the development of phage-resistant staphylococci in broth. (2) Sulfanilamide, sulfapyridine, sulfadiazine, and sulfanilyl-guanidine may delay the secondary growth of *S. dysenteriæ* (Shiga) and *E. coli* in broth containing bacteriophage. (3) The phage-resistant bacilli were found to be susceptible to the bacteriostatic action of sulfamido compounds.

The author wishes to express his sincere appreciation to Dr. Philip Levine, Newark, New Jersey, for Shiga bacteriophage; to Dr. E. K. Marshall, Jr., Johns Hopkins University, for sulfanilyl-guanidine, and to Dr. David A. Bryce, Lederle Laboratories, for sulfadiazine.

13025

Plasma Clotting Time and Serum Calcium of Patients Recovered from Attacks of Coronary Thrombosis.

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It has been suggested that it is possible to predict among those patients who have undergone surgical operation those who are likely to have thrombosis or embolism by detecting a decreased clotting time of the blood plasma.[1] The same authors also thought that it might be possible to alter the clotting time of the blood by appropriate diet and by administering sodium thiosulfate. In view of the above considerations, it seemed worth while to investigate the possibility that patients who are subject to attacks of coronary thrombosis might show a tendency to a more rapid clotting of the blood. Sixteen patients recovered from such attacks were compared with 14 of the same age group who did not have any complications of the heart:

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[3] Marshall, E. K., Braton, A. C., Edwards, L. B., and Walker, E., *Bull. Johns Hopkins Hosp.*, 1941, **68**, 94.

[1] Bancroft, F. W., Stanley-Brown, M., and Quick, A. J., *Am. J. Surg.*, 1935, **28**, 648.

Methods. Owing to the fact that the plasma clotting time is influenced by what appear to be negligible factors, it is necessary to describe rather fully the experimental technic.

All tubes and syringes were brushed with soap and water, rinsed with cleaning solution, then with tap water repeatedly, and finally with distilled water. Clean syringe needles were boiled in distilled water and dried in an oven.

0.5 cc of 0.1 M sodium oxalate solution was measured into a 15 cc centrifuge tube. About 12 cc of blood were drawn as rapidly as possible and with special care to avoid contamination with tissue juice. 4.5 cc of blood were immediately transferred to the centrifuge tube by running it down the sides so as to prevent injury to the cells. The tube was then quickly inverted over a rubber stopper to mix the blood with the oxalate solution and centrifuged at about 1,400 r.p.m. for 5 minutes, after which the plasma was removed from the cells with a capillary pipette. The remainder of the blood from the syringe was centrifuged and a calcium determination made on the serum by the Clark-Collip[2] modification of the Kramer-Tisdall method.

The thromboplastin was prepared according to the directions of Quick.[3] The brains of 5 freshly killed rabbits were ground together, spread on gauze and dried. The emulsion was freshly prepared each day by leaching 1 piece of the gauze (representing 1 g of the fresh brain) in 10 cc of normal NaCl solution.

For the determination of the plasma clotting time, 2 drops of the oxalated plasma were delivered into a 13 x 100 mm test tube and warmed for 1 minute in a 37°C constant temperature water bath. Two drops of 0.1 M calcium chloride solution, a bottle of which was kept warm in the water bath, were then added and the stop watch instantly started by an assistant. The tube was kept in the bath for 1 minute from the time the calcium chloride solution was added, then taken out for inspection, as 5-second intervals were called, until the formation of the clot occurred. At least 3 tests were made of each specimen. If these 3 tests checked within 5 seconds, the average was designated the plasma clotting time. If there was a greater difference in the time for the first 3, the test was repeated until close checks were obtained in triplicate.

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[2] Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, 1925, **63**, 461.

[3] Quick, A. J., *Am. J. Physiol.*, 1935-36, **114**, 282.

The prothrombin time was determined by adding 2 drops of plasma and 2 drops of the thromboplastin emulsion to the small tube and warming in the water bath for 1 minute. Two drops of 0.1 M calcium chloride solution were then added and the warming continued for 10 seconds by the stop watch. When the 10-second time was called by the assistant, the tube was removed and the clotting time was recorded as observed. The average of 3 readings which checked closely was taken as the prothrombin time.

TABLE I.
Plasma Clotting Time and Serum Calcium of Controls and Cases of Thrombosis.

Determination	Max.	Min.	Mean	Max.	Min.	Mean	No. of cases
	sec.			mg per 100 cc			
Prothrombin time	19.7	16.2	17.4				14 controls
Plasma clotting time—							
appearance of fibrin	95	80	83.3				
formation of clot	100	86	90.7				
Serum calcium				10.8	9.9	10.5	
Prothrombin time	19.8	16.8	17.9				16 thrombosis
Plasma clotting time—							
appearance of fibrin	91	78	82.2				
formation of clot	110	81	95.4				
Serum calcium				11.5	10.2	10.9	

Results and Conclusions. The data presented in Table I show that there was no significant difference in the prothrombin time, in the plasma clotting time as indicated by the appearance of fibrin, or in the serum calcium content of the blood serum of persons who had suffered attacks of coronary thrombosis when compared with a group of controls. There was a difference in the interval between the appearance of fibrin and the formation of a solid clot of 7.4 seconds in the control series and of 13.2 seconds in the group of thrombosis cases. This difference was found to be significant when analyzed by the t test of Fisher.[4] Since the time for the appearance of fibrin was practically the same for the 2 groups, this means that the blood of the thrombosis patients did not form a solid clot so readily as did that of the controls. Obviously, this difference indicates a tendency to a longer and not a shorter coagulation time of the blood of patients who have had attacks of coronary thrombosis.

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[4] Fisher, R. A., *Statistical Methods for Research Workers,* London and Edinburgh, 6th Edition, 1936.

13026

Para-Aminobenzoic Acid and Dopa Reaction.

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Melanoblast pigmentation has been considered by Bloch[1] to be due to a specific dopa oxidase causing the formation of melanin by its action on dopa (3,4-dihydroxy-phenyl alanine). Since p-aminobenzoic acid has chromotrichial activity,[2, 3] it probably plays a rôle in the formation of melanin which can be demonstrated experimentally. Therefore, the influence of this acid was studied in the reactions yielding melanin and catalysed by the readily available potato tyrosinase, namely in tyrosine-tyrosinase, dopa-tyrosinase, and catechol-tyrosinase systems. Other substances were investigated under comparable conditions, such as, *e. g.*, sulfanilamide, the bacteriostatic effect of which is nullified[4, 5, 6] by p-aminobenzoic acid; aniline, the simplest aromatic amine; and calcium pantothenate, because its curative effect has been observed in gray-haired animals reared on pantothenic acid-deficient diets.[7, 8]

In the experiments recorded in Table I, tyrosine and tyrosinase passed through the typical red[9] stage before producing the black, flocculent melanin precipitate. When p-aminobenzoic acid was present, the intermediate red phase was not developed, the black melanin precipitate was not formed, but a brownish reaction mixture was produced. Similar phenomena were observed when sulfanilamide was employed in place of the acid. The experiments were repeated with dopa-tyrosinase as well as with catechol-tyrosinase and comparable data obtained. However, as is well known, the speed of reactions is more rapid in the dopa-tyrosinase and in the catechol-tyrosinase than in the tyrosine-tyrosinase system. Furthermore, the color changes observed in the catechol experiments were not identical with those in the other systems. Nevertheless, the influence of p-aminobenzoic acid was definite, and it appeared to be the same in the case of the 3 substrates, tyrosine, dopa, and catechol. This latter observation was to be expected, the first step in the oxidation of tyrosine by tyrosinase being the formation of dopa, as pointed out by Raper.[10]

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[1] Bloch, B., *Z. physiol. Chem.*, 1917, **98**, 226.

[2] Ansbacher, S., *Science*, 1941, **93**, 164.

[3] Martin, G. J., and Ansbacher, S., *J. Biol. Chem.*, 1941, **138**, 441.

[4] Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.

[5] Landy, M., and Wyeno, J., Proc. Soc. Exp. Biol. and Med., 1941, **46**, 59.

[6] Strauss, E., Lowell, F. C., and Finland, M., *J. Clin. Invest.*, 1941, **20**, 189.

[7] Unna, K., and Sampson, W. L., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 309.

[8] György, P., and Poling, C. E., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 773.

[9] Raper, H. S., and Wormall, A., *Biochem. J.*, 1925, **19**, 84.

TABLE I.
Tyrosine-Tyrosinase System.*

Addition compounds	Intermediate phase		Reaction product in 18 hr	
	Color	Time, min.	Color	Precipitate
None (control)	red	30	black	flocculent
Tryptophane	red-purple	35	"	"
Calcium pantothenate	" "	35	"	"
Benzoic acid	red	90	"	"
o-Cresol	red tint	90	"	"
Ascorbic acid			red	none
Cysteine			faint red	"
p-Hydroxy-benzoic acid	red	30	brownish with red tint	"
p-Hydroxy-phenyl acetic acid	"	30	" " " "	"
Hydroquinone	"	35	brownish	"
p-Aminobenzoic acid	faint brownish	35	"	"
Sulfanilamide	" "	40	" with green tint	"
Aniline	red-gray	90	slight-brown	"

*The pH of 20 mg of addition compound and 20 mg of tyrosine in 10 ml of saline solution was adjusted to 7.0; then 10 ml of phosphate buffer and potato slices (or juice) were added.

The data as a whole seem to show conclusively that, under identical experimental conditions, calcium pantothenate has no influence and that p-aminobenzoic acid and sulfanilamide are effective in modifying melanin formation. It appears, therefore, that an enzyme system has been found in which the 2 aromatic amines attack at the same point. This is particularly interesting, because p-aminobenzoic acid inhibits the antibacterial action of sulfanilamide,[4, 5, 6] a fact which suggests a common point of attack for the 2 substances in bacteria systems.

Raper and Wormall[9] have shown that melanin formation occurs in 3 stages, that the enzyme is important in the first part, the result of which is a red pigment, and that the 2 subsequent phases, involving decolorization of the red pigment and oxidation to melanin, do not require an enzyme. Since the oxidation of tyrosine by tyrosinase does not go through the characteristic red phase when p-aminobenzoic acid or sulfanilamide is present in the dopa enzyme system, it appears that aromatic amines can play a rôle in the non-enzymatic portion of the process. It may be that the intermediate phase is not observed, because the amino group of p-aminobenzoic acid reacts with the red substance (halochrome) to give soluble anilino-like compounds which undergo auto-oxidation to a brownish melanin more soluble than that derived from tyrosine alone. Pugh and Raper[11] did not observe aniline to form an anilino compound with tyrosine oxidized by tyrosinase, but isolated anilino compounds when catechol or p-cresol was oxidized by tyrosinase and oxygen. Moreover, they found that aniline alters melanin formation, an observation confirmed by the data of Table I, since no black pigmentation occurred when tyrosine was acted upon by tyrosinase in the presence of the aromatic amine.

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[10] Raper, H. S., *Biochem. J.*, 1926, **20**, 735; 1927, **21**, 89.

In view of the above, it is conceivable that p-aminobenzoic acid modifies in the animal organism the type of melanin produced from the oxidation of dopa by dopa oxidase, an enzyme which has been found in skin by Bloch and Schaaf.[12] The nature of this enzyme is not clearly defined. Whether it will eventually be found to be a specific dopa oxidase[1] or an inhibited tyrosinase,[13, 14] is immaterial as far as the data presented are concerned, because p-aminobenzoic acid appears to enter the non-enzymatic portion of the reactions leading to the formation of melanin.

Summary. Para-aminobenzoic acid modifies the formation of melanin; sulfanilamide has an apparently similar effect; under identical conditions, calcium pantothenate has no influence.

13027

Inability of Desoxycorticosterone to Maintain Lactation.*

ROBERT GAUNT.

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The following facts have established the adrenal cortex as an important factor in lactation: (1) adrenalectomized rats do not

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[11] Pugh, C. E. M., and Raper, H. S., *Biochem. J.*, 1927, **21**, 1370.

[12] Bloch, B., and Schaaf, F., *Biochem. Z.*, 1925, **162**, 181.

[13] Raper, H. S., *Physiol. Rev.*, 1928, **8**, 245.

[14] Figge, F. H. J., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 269.

* This work was aided by a grant from the American Philosophical Society. We are indebted to Dr. Erwin Schwenk, the Schering Corporation, for the desoxycorticosterone acetate (cortate) used here.

lactate normally.[1-5] This deficiency can be corrected by cortin but only if relatively large doses are given;[6] (2) changes are apparent histologically in the mammary glands of lactating, adrenalectomized rats;[7] (3) the induction of an experimental lactation is facilitated in rats by cortin injections;[8] (4) the hypophysectomized guinea pig can be made to lactate with purified lactogenic hormone only if cortin, adrenotropin or salt is concomitantly given.[9] The latter point may not be true of the hypophysectomized rabbit.[10] Brownell, Lockwood and Hartman believed that the lactation-supporting factor of the adrenal cortex, which they designated *cortilactin,* was chemically separable from the life-maintaining factor.[3] The study of crystalline cortical substances is of obvious importance in elucidating the physiology of the above-mentioned phenomena.

This report concerns the effect of desoxycorticosterone acetate (DCA) on the lactation of rats adrenalectomized within 24 hours after delivery. The growth rate of the suckling young was used as an index of lactation. All litters were reduced to 6 each. Although the growth curves included here are shown for the first 30 days, they serve as a measure of lactation only up to 15 to 20 days, at which time the young began to eat. Well-nourished litters of normally lactating mothers began to eat earlier that the various dwarfed litters. Treatments were usually discontinued at 20 days if the litters lived that long. I. The normal growth rate for this rat colony is shown in curve 1 for 15 unselected litters, reduced to 6 young each. The mortality from all causes was 5% and probably in no case was due to lactation failure. II. In 7 untreated adrenalectomized mothers lactation was sub-normal but no deaths of young occurred before 15 days and 34% were raised to weaning (curve 3). This result is somewhat better than we previously found, probably because the adrenalectomies were delayed until after parturition. The mothers lost an average of only 5% of their body weight while

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[1] Carr, J. L., Proc. Soc. Exp. Biol. and Med., 1931, **29**, 131.

[2] Gaunt, R., *Am. J. Physiol.*, 1933, **103**, 494.

[3] Brownell, K. A., Lockwood, J. E., and Hartman, F. A., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 783.

[4] Britton, S. W., and Kline, R. F., *Am. J. Physiol.*, 1936, **115**, 627.

[5] Tobin, C. E., Proc. Soc. Exp. Biol. and Med., 1939, **41**, 599.

[6] Gaunt, R., and Tobin, C. E., *Am. J. Physiol.*, 1936, **115**, 588.

[7] Levenstein, I., *Anat. Rec.*, 1937, **67**, 477.

[8] Reece, R. P., Proc. Soc. Exp. Biol. and Med., 1939, **40**, 25.

[9] Reviewed by Nelson, W. O., and Gaunt, R., *Cold Spring Harbor Symp. Quant. Biol.*, 1937, **5**, 398.

[10] Fredrikson, H., *Acta Obst. et Gynec. Scand.*, 1939, **19**, Suppl. 1, 1.

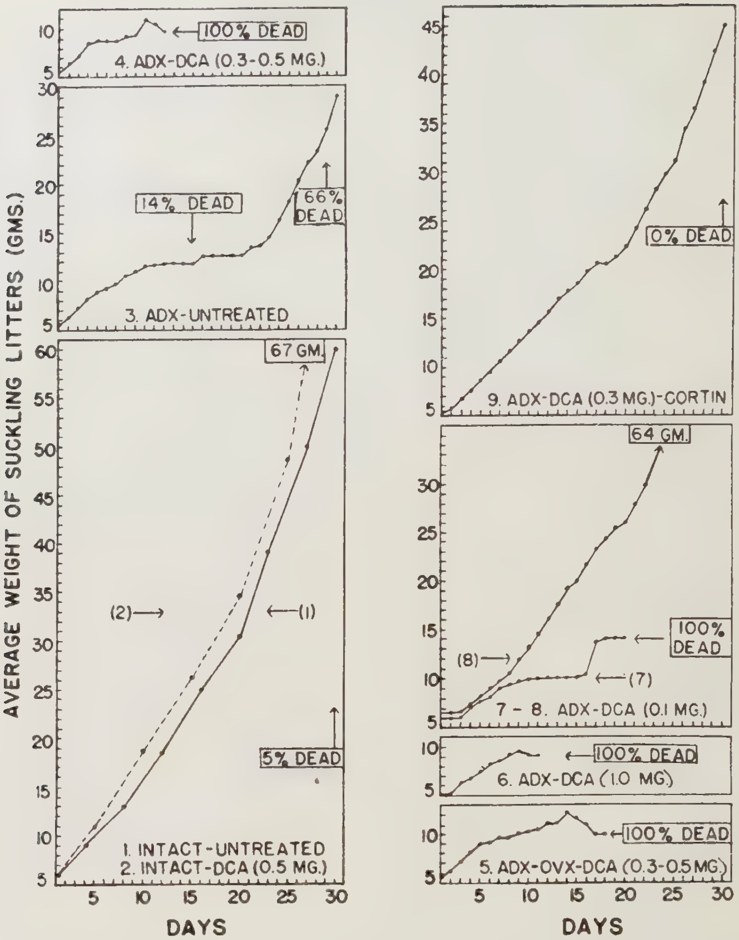

FIG. 1.

Average growth curves of rat litters from mothers receiving various treatments. Curve 1 is the average of 15 litters; curve 2, 4 litters; curve 3, 7 litters; curves 4, 5 and 9, 4 litters each; curve 6, 2 litters; curve 7, 1 litter; curve 8, 3 litters. Abbreviations are as follows: Adx.—adrenalectomized; Ovx—ovariectomized; DCA—desoxycorticosterone acetate.

lactating. Shortly after lactation stopped adrenal insufficiency became more severe and all but one mother died.

Previous work showed that cortin, given in adequate dosage to such adrenalectomized animals, supports a completely normal lactation.[6] III. DCA was given to a total of 14 adrenalectomized mothers in doses ranging from 0.1 to 1.0 mg per day (curves 4-8). Of this group 13 litters died, and one, anomalously, grew normally (curve 8), although the mother later died of adrenal insufficiency. The failure of lactation in these cases occurred despite freedom from all obvious symptoms of adrenal insufficiency. All but one of the 14 animals gained weight while lactating, with average increments as follows: those shown in curve 4 gained 8%; curve 5, 17.8%; curve 6, 12%; curve 7, 4%; and curve 8, 15%. Normal untreated mothers (curve 1) gained 4.2% during the first 20 days of lactation. Thirteen of the mothers died after treatment was discontinued.

It is clear that DCA will not support lactation. There is also a suggestion in these results, first, that lactation is worse instead of better with the larger doses of DCA, and, secondly, that litters of untreated mothers fared better than those of treated mothers, *i. e.*, that DCA inhibited lactation. We do not consider that the differences were great enough to establish this as fact, but if it eventually proves to be true a reason may be found in the observation that DCA is a mammary-growth stimulant,[11] and therefore might be expected to inhibit lactation when given in proper dosage.[12]

IV. There was no significant influence of 0.5 mg DCA per day on the lactation of intact rats (curve 2), indicating that if this substance is a lactation inhibitor its effects are counteracted (at this dose level) by other cortical secretions. The same conclusion is indicated by the near normal response of adrenalectomized mothers given DCA plus 2 cc per day of an old and relatively weak batch of cortin (curve 9).

V. The dose of estrogen needed to inhibit lactation is about 10× greater in castrate than intact rats.[13] We thought, therefore, that ovarian removal might modify the response of adrenalectomized mothers given DCA. A comparison of curves 4 and 5 suggests that possibly the castrates lactated a little better than animals with ovaries intact. but the difference was of questionable significance.

Summary. The necessity of adrenal cortical secretions for the support of lactation in rats was confirmed. Desoxycorticosterone acetate, unlike adrenal cortical extract, was of no benefit at all in correcting this deficiency, and may have further depressed lactation.†

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[11] Van Heuverswyn, J., Folley, S. J., and Gardner, W. U., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 389.

[12] Folley, S. J., *Lancet*, 1941, **1**, 40.

[13] Edelmann, A., and Gaunt, R., *Physiol. Zool.*, in press.

† Since the above experiments were completed tests have been made, in collaboration with Dr. E. C. Kendall, on the lactation-maintaining potency of other adrenal compounds. This work is still in progress but the following facts have been established: Kendall's compound A (dehydrocorticosterone) and compound E (17-hydroxy-11-dehydrocorticosterone) were about equally effective, and in daily doses approximating 0.5 mg maintained a growth rate in suckling young which approached but did not reach normal (9 of 10 litters survived). Kendall's cortin was distinctly more effective than his amorphous fraction when given in doses that were of equal potency in maintaining adrenalectomized dogs.

13028

Induction of Female Behavior in Male *Anolis carolinensis* with Testosterone Propionate.*

G. K. Noble and B. Greenberg.

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In the course of an earlier experiment,[1] dealing with the effects of testosterone propionate upon the urogenital system and sexual behavior of the lizard, *Anolis carolinensis,* we observed a single case of an immature, testosterone-treated, castrate male (38 mm in body length) which displayed female as well as male mating behavior. The purpose of the present report is to describe the effects of testosterone propionate upon the sexual behavior of a large series of immature male *Anolis.*

Experimental Procedure. Fifty-two immature male *Anolis carolinensis* (average length 48.4 mm from snout to anus) were divided into 3 experimental groups. Thirty-one were subjected to partial hypophysectomy, 12 were castrated and 9 served as an unoperated control group. Hypophysectomy was attempted in order to secure morphological data, which will be reported separately. Castration was performed to eliminate normal production of testicular hormone.

Pituitary insult was not followed by obvious postoperative shock. Thirteen to 15 days after operation, 15 partially hypophysectomized males received subcutaneous implants of testosterone propionate pellets.† The remaining 16 lizards of this group received no hormone therapy. Recovery appeared complete 3 days after castration and 6 castrated males were then implanted with testosterone propionate pellets, while 6 other castrates were kept as untreated controls. Five unoperated males were given similar implants and 4 intact animals were untreated. The pellets were removed, dried and reweighed at the end of the experiment. At insertion, the pellets

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* Aided by grants from the Committee for Research in Problems of Sex, National Research Council, and from the Josiah Macy, Jr., Foundation.

The experiment was planned together with and completed under the direction of the senior author. Manuscript preparation and final interpretation of results are the responsibility of the junior author.

[1] Noble, G. K., and Greenberg, B., Proc. Soc. Exp. Biol. and Med., 1940, **44**, 460.

† We are indebted to the Ciba Pharmaceutical Products, Inc., for the testosterone propionate (perandren) utilized in this study.

ranged in weight from 1.11 to 3.71 mg, the average weight being 2.62 mg. The amounts apparently absorbed after 26-29 days of treatment varied from 0.63 to 1.54 mg with an average of 1.02 mg. However, there were some losses of hormone in handling and in attachment to the skin.

The 52 lizards were kept in 5 similarly arranged cages (35 inches by 33 inches by 42 inches). Each cage contained implanted and non-implanted males which had received the same operative treatment. Large Spider Lilies *(Crinum)* and crossed tree branches provided climbing and hiding places. The cages were located in a sunlit greenhouse with a constant temperature of 28°C. Diet consisted of flies and mealworms, and water was provided by sprinkling the cages 3 times daily. Behavior was observed constantly from 9 a.m. to 4 p.m. on 5 week days and occasionally on Saturdays and Sundays.[‡] The observation period extended from October 28, 1940 through November 17, 1940.

Results and Discussion. Two forms of social behavior are observed in normal *Anolis:* fighting and sexual activity.[§] In the former, one lizard is dominant over all other individuals in the group. Dominance is manifested by a very clear postural attitude; subordination by a series of characteristic head bobs. In mating, the active male, bobbing rapidly, displays his dewlap (a colored gular fan), struts toward the female and attempts to secure a neck grip. The female in full estrus submits to copulation without struggle or running away, and flexes her neck as the male approaches. The complete copulation position consists of the male's neck grip, body of the male arched over that of the female and his pelvic region, tail and legs arranged in a characteristic grip around the female's tail base. In the discussion which follows, the terms "dominant" and "subordinate" will be used to describe fight relationships, while "male" and "female behavior" will refer to mating.

Many of our testosterone propionate-treated males, whether partially hypophysectomized, gonadectomized or unoperated, reacted sexually both in typical male and in typical female fashion, while their respective untreated controls were completely inactive.

Although each cage contained an equal number of control and implanted lizards, the controls were never observed to exhibit masculine sexual reactions. Thirty-nine matings occurred in cages containing partially hypophysectomized animals. Implanted lizards played the masculine rôle in all of these encounters, and in 37 of the 39 instances testosterone-treated animals served as the submissive partner. In the cage containing castrated males 10 copulations were recorded. In each case only implanted lizards were involved. The 9 matings observed in the cage containing unoperated males were all between testosterone-treated lizards. Nonimplanted lizards participated in only 2 of the 58 copulations recorded. In both of these instances the masculine rôle was taken by a treated male.

[‡] Mr. H. Bloom and Miss S. Dushkind assisted in observing and recording behavior. The personnel of Works Progress Administration, Official Project No. 165-1-97-8 (WP-10) aided in animal maintenance and preparation of materials.

[§] A full account of the social behavior of this species is in preparation by the junior author.

The estrous type of neck bend, characteristic of female sexual behavior was observed 10 times in the testosterone-treated, partially hypophysectomized males (4 individuals, one reacting 5 times); and one treated castrate displayed this behavior 4 times. No neck bends were observed on the part of unoperated testosterone-treated males. However, in a further experiment at present in progress, 2 intact males (37 and 39 mm in size) have been observed to bend their necks after implantation with testosterone propionate pellets. No neck bends were observed in any of the control groups.

To illustrate the dual nature of the response to testosterone, we quote directly from experimental records. Male 3 reacted to 2 other testosterone-treated males by alternately displaying male and female sexual behavior.

1:15—Cage G—Male 57 approaches male 3 and male 3 bends neck (female behavior). Male 57 courts male 3 who stands with neck bent. Male 57 moves away.

2:30—Cage G—Male 57 courts male 10. Male 3 is a foot away, bobs subordinately and approaches half the distance. Male 10 moves close to both other males. Male 3 courts and shows dewlap (male behavior). Male 57 shows a combination of fight and courtship toward male 3 who bobs subordinately and bends neck (female behavior). Male 57 does not mate. Male 10 moves past male 3 who again courts male 10 and approaches him in typical strut (male behavior). Male 57 comes over in fight position, approaching male 3 very closely. Male 3 bobs subordinately and bends neck (female behavior).

The following day male 57 mated male 9, another testosterone propionate-treated male. After the mating the following behavior was observed:

12:45—Cage G—Male 3 approaches male 57. The latter displays in fight challenge and male 3 bobs subordinately. Male 57 approaches in fight attitude and again male 3 bobs subordinately. Male

57 rests his chin on male 3 and male 3 stands with neck bent (female behavior). Male 57 displays in fight challenge and as male 3 remains with neck bent, moves away.

Male 3 jumps at male 10 and grips at neck (male behavior). Male 10 struggles at first, then stops. Male 57 approaches and attacks the pair in tense fight posture. Males 3 and 10 fall from the leaf, emerging again from under a lower leaf. Male 57 attacks and nips at the pair, biting male 3 across the head. This breaks the pair. At 1:10, male 3 courts male 2 (a control) who escapes (male behavior).

This case clearly shows that a dominance relationship existed between male 57 and male 3, and although 3 was sexually active in the masculine direction, his reaction to 57 was of the estrous female type.

The implanted males were observed to fight much more frequently than the controls. Ninety-nine fights occurred in cages containing partially hypophysectomized lizards. Implanted animals won 97 of these encounters; untreated males won twice. Eight fights occurred in the cage of castrated males, and in each case the winner was a testosterone propionate-treated lizard. One hundred and one of 107 fights occurring among intact males were won by hormone-treated animals, and in 6 cases the victor was a control lizard. The loser in various fights was sometimes a treated and sometimes a control male. Although no statistical data are available it is probable that in the majority of cases the loser as well as the victor was a hormone-treated male. This conclusion is indicated by the observation that the aggressive nature of implanted lizards tended to involve such animals in the majority of encounters. Controls were apt to avoid conflict by abrupt withdrawal before any real attack occurred.

In each of the 5 cages, one or 2 of the testosterone-treated males fought and won most of the encounters. In all but one of these cases the dominant male also mated most frequently. Table I indicates the correlation between dominance and frequency of masculine copulatory pattern.

In the one exception, male 3 in cage F fought most often (a total of 56 times), and courted most often (11 of 24 courtships in this cage), but was never observed to mate. Although obviously stimulated to court and fight, he did not complete the pattern even though other treated males stood before him with necks flexed. This lizard repeatedly broke up the mating of other residents in his cage by

TABLE I.

Relation Between Dominance in Fighting and Copulation in Testosterone-treated Immature Male *Anolis carolinensis*.

Group	Cage	Male No.	Fights as Dominant	Total fights (all individuals)	%	Matings as masculine partner	Total matings (all individuals)	%
Partially hypophysectomized males	F	3	56	65	86	0	5	—
	G	57	19	22	86	8	16	50
	*H	9	5			5		
		10	6			8		
Total	H		11	12	92	13	18	72
Castrate males	J	4	6	8	75	7	10	70
Intact males	D	1	87	107	81	6	9	67
Totals		6	179	214	83	34	58	59

*Two males were successively dominant in this cage and are therefore considered together.

fight attacks upon pairs in preliminary neck-grip position. His behavior markedly reduced courtship activity in Cage F. One factor underlying this dominance was his size, 54 mm, the largest male in the cage. On one occasion, however, male 3 assumed the feminine rôle in a copulation with a smaller treated male (48 mm in body length). The behavior of this individual indicates the importance of other factors in producing the complete copulatory pattern, such as possible differences in threshold of response of the central nervous system to testosterone propionate.

As indicated in Table I and by the excerpts from our experimental records, male behavior in copulation appears to be correlated with dominance in fighting, and this supports our previous conclusion[1] with reference to females: that in any one testosterone propionate-treated individual, the assumption of male or female sex behavior is correlated with position as dominant or subordinate in fights among the group.

Prolonged testosterone propionate treatment appeared to have an effect upon the male copulatory pattern. In 20 matings (15 copulations by 8 partially hypophysectomized males and 5 copulations by 3 castrated males), it was observed, upon assumption of the complete mating posture, that this posture was maintained for periods of 10 minutes to an hour if the partner managed to pull away during the copulation. A male showing this behavior could be handled and examined without breaking the tension of his body, and in running away he might even maintain the bent tail-region. Normal males do not continue in mating position if disturbed. Evidently testosterone reinforces the reflexes involved in the copulation posture.

Summary. Testosterone propionate, introduced in pellet form into intact, castrated or partially hypophysectomized immature male *Anolis carolinensis,* elicits both male and female sexual behavior, the latter including submission to copulation and display of a distinctive neck reflex. Testosterone-treated males fight more actively than controls, and the assumption of male or female behavior is apparently related to position as dominant or subordinate in fights among the group. Testosterone affects the reflexes involved in the copulation posture so that they are maintained in exaggerated form after the escape of the partner.

13029 P

Bile Acids and the Pulmonary Tumor Incidence in A Strain Mice.

L. W. Law.* (Introduced by C. C. Little.)

From the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

The principal bile acids of the higher vertebrates are hydroxy derivatives of cholanic acid, a compound which can be obtained *in vitro* from sterols by degradation. The bile acids as well as sterols have condensed carbon ring molecules to which are attached side-chains in a position such that a new 6-membered ring can be formed so as to give the carcinogenic 1:2-benzanthracene ring system without any molecular rearrangement or migration of groups within the molecule. In 1934 it was shown[1] that such a ring closure to the 1:2-benzanthracene system had occurred in the formation of dehydronorcholene from desoxycholic acid. Upon dehydrogenation of this product methylcholanthrene, a highly carcinogenic substance, was formed. Later[2] it was shown that methylcholanthrene could be obtained from cholic acid, the chief acid of human bile.

These discoveries led to tests for carcinogenic properties of the bile acids with conflicting results. Application of desoxycholic acid in alcohol or benzene to the skin in a large series of mice gave completely negative results. Injections *sub cutem* of 70 mg desoxycholic acid in sesame oil produced sarcomata in 3 of 10 mice at a late date.[3] This experiment was repeated using the C3H strain of mice, a very suceptible strain to induction of subcutaneous tumors by carcinogens. Seven spindle-cell tumors were obtained in 208 days, the first appearing 155 days after receiving a total of 28 mg in 6 injections. Shear obtained completely negative results following injections *sub cutem* of both cholic and desoxycholic acids. Ghiron reported that transplantable fibrosarcomata were induced in a high percentage of rats and mice following injections of desoxycholic acid, however no further details of this work have been published.

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* Finney-Howell Foundation Medical Research Fellow.

[1] Cook, J. W., and Haslewood, G. A. D., *J. Chem. Soc.*, 1934, 428.

[2] Fieser, L. F., and Newman, M. S., *J. Am. Chem. Soc.*, 1935, **57**, 961.

[3] Cook, J. W., Kennaway, E. L., and Kennaway, N. M., *Nature*, 1940, **145**, 627.

The purpose of this preliminary report is to record the response of lungs of A strain mice to 2 bile acids[†] injected subcutaneously. The Jackson Laboratory A strain has a high incidence of pulmonary tumors.[4] Spontaneous tumors in mice under 9 months of age usually appear as single nodules just underneath the pleura, whereas multiple nodules appear very early in mice treated with carcinogens.[5] Equal numbers of pedigreed males and females were used and kept separate. One experimental group was given a total dosage of 10 mg cholic acid dissolved in olive oil. The first injection was given at the age of one month and 3 subsequent injections were given within the following 2 months. A second experimental group was given the same dosage of sodium desoxycholate. The animals were killed at 9 months of age, 8 months following initial injections. Lung nodules were looked for grossly under the dissecting microscope and histological confirmation was made of nodules.

From Table I it can be seen that sodium desoxycholate has increased the pulmonary tumor incidence ($P < .01$) and increased the number of nodules found in the lung. In this experimental group there appeared a fibrosarcoma in an animal 7 months of age. Spontaneous sarcomata are rare in A strain mice and none have been recorded as appearing at this age. There was no effect of cholic acid on the pulmonary tumor incidence.

TABLE I.
Response of Lungs of Strain A Mice to Bile Acids.

Treatment	No. mice	No. lung tumors	% lung tumors	Mean No. nodules in lung
Sodium desoxycholate	20	16*	80.0	3.0
Cholic acid	30	14	46.7	1.4
Controls	38	18	47.3	1.9

*One mouse killed at 8 months—fibrosarcoma.

[†] Supplied through the courtesy of Dr. Oliver Kamm, Parke, Davis & Co.

[4] Bittner, J. J., *Pub. Health Rep.*, 1939, **54**, 380.

[5] Andervont, H. B., *Pub. Health Rep.*, 1937, **52**, 304.

Mice of strains C3H, C57 black, and A^W, known to be among the most susceptible to subcutaneous growths after application of carcinogens, have not as yet (10 months) given any subcutaneous tumors following similar treatment with cholic acid and sodium desoxycholate.

A study of the effect of larger doses of desoxycholic acid on pulmonary tumor incidence in A strain mice is being continued.

Summary. A significant increase in the incidence of pulmonary tumors is recorded in A strain mice following subcutaneous injection of 10 mg sodium desoxycholate. No such response was indicated following similar treatment with cholic acid.

13030 P

Histophysiological Study on Activity of Stellate Cells of Frog Liver.*

RUDOLF HÖBER AND JOSEPHINE HÖBER.

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Activity of stellate cells in the liver ordinarily is tested by their capacity to incorporate microscopic or submicroscopic particles, e. g., colloidal trypan blue. In isolated perfused frog liver this uptake is shown to be an active process by the fact that it is reversibly suppressed by addition to the perfusion fluid of an anesthetic or of iodoacetic acid.[1] Also, the color uptake is stopped by perfusing, for a short time, with Ringer's solution, but reappears upon the addition of serum or of various protein bodies.[2, 3, 1] While studying the influence of surface-active and nonpolar-polar organic compounds on erythrocytes, muscles, nerves, liver, kidney,[4] we were reminded of the statement of A. J. Clark[5] that the strikingly beneficial effect of serum on the heart of a frog, which has become

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* This work has been supported by grants from the Ella Sachs Plotz Foundation and from the Faculty Research Committee, University of Pennsylvania. Activating compounds were supplied by E. I. du Pont & Company, by Riedel-de Haen and by the American Cyanamid Corporation.

[1] Ferrari, R., and Höber, R., *Pflüger's Arch.*, 1933, **232**, 299.

[2] von Janscó, N., *Z. Exp. Med.*, 1928, **61**, 63.

[3] Höber, R., and Titajew, A., *Pflüger's Arch.*, 1929, **223**, 180.

[4] Höber, R., *Cold Spring Harbor Symposia*, 1940, **8**, 40.

[5] Clark, A. J., *J. Physiol.*, 1913, **47**, 66.

hypodynamic by a prolonged perfusion with Ringer, is due chiefly to presence of minute amounts in the serum of lipoids and soaps. We decided to study the influence of physico-chemically similar compounds upon the activity of the stellate cells.

Methods. The liver of female frogs was perfused through the abdominal vein with buffered Ringer solution (pH 7.5). In general, after 30 min. 2% ox serum was added, 10 min. later 0.02% trypan blue and a specified amount of a test substance were added. After another 45 min. the liver was placed in formalin and cut by a freezing microtom. In each experiment 2 to 4 frogs of approximately the same weight and belonging to the same group were compared. The same fresh stock of serum was used for all the perfusions in each experiment to avoid error resulting from varying amounts of activating substances in the sera.

Results. With livers identically treated the uptake of color varies very much, mainly because of seasonal variations. With an abundant uptake, the color appears in a fairly continuous blue network of the blood capillaries surrounding the unstained islets of liver cells. This network consists of irregularly shaped groups of stellate cells, which show a rather homogeneous coloration. With a small uptake one finds separate longer or shorter blue bars or rods. In general, the nuclei are unstained. A comparison of sections from the same set of livers shows that after Ringer perfusion almost no blue color can be detected. Addition of serum is followed by a definite coloration, which however differs according to the amount of activating matter being present in the serum. Further addition in rising concentrations of surface-active nonpolar-polar compounds results in increased color uptake which is indicated either by a stronger coloration of the stellate cells or by the fact that with higher concentrations the cells begin to swell and that the dye spreads over the enlarged cell body.

Bile Salts. Due to their marked nonpolar-polar molecular configuration as well as to their powerful choleretic activity the bile salts were expected also to influence the behavior of the stellate cells. As a matter of fact, they increase the amount of trypan blue deposited more consistently than any of the tested substances. Therefore, the aforementioned blue network is particularly conspicuous. An increase of the cell volume can contribute to the distinctness of the picture. This is most evident with about 3×10^{-5} molar taurocholate or glycocholate. Beyond this concentration, the areas showing the diffuse blue coloration are enlarged to a greater extent and appear to be formed by aggregates of more greatly swollen cells and by irregularly formed pale-blue cell rests, parts of which extend into the lumina of the broader capillaries. At this stage of structural alteration one meets more or less marked amounts of nuclear staining, even in the liver cells proper.

Similar results are obtained with other organic nonpolar-polar substances, by means of which a dispersing effect in the colloidal systems can be produced and, therefore, the normal properties of the surface film of cells can be altered. Among such compounds investigated, detergents and wetting agents (aerosols, sulphated fatty acids, hexylresorcinol) have proved effective.

Discussion. According to the histological effects described, the substances tested in this study can be compared to cytolytics, although such a classification would appear to deprive the results of their physiological meaning. However, cytolysis is only the end effect of a range of structural and ultrastructural changes, which, at their very beginning, are reversible and are indicative of changes falling into the range of physiological manifestations. This has been found already in earlier experiments with the Ringer perfused frog liver insofar as cytolytics have been shown to increase the secretory transport of dyestuffs in low concentrations and to abolish it in higher concentrations.[6]

13031 P

Necrosis, Cirrhosis and Cancer of Liver in Rats Fed a Diet Containing Dimethylaminoazobenzene.*

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It was recently reported[1] that dietary liver injury (necrosis, cirrhosis) seen in rats fed a synthetic ration is determined in great part by the absence of the lipotropic activity of casein. These changes can be prevented to a large extent by the addition of casein to the diet, but this is accomplished more effectively by the combined oral administration of cystine and choline.

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[6] Höber, R., and Moore, E., *J. Gen. Physiol.*, 1939, **23**, 191.

[*] The contents of this paper have been presented by one of us (P.G.) as part of two lectures, one given on Feb. 12, 1941, before the Society of the Sigma Xi, Western Reserve Chapter, and the other on March 4, 1941, before the Duke Medical Society, Durham, North Carolina.

[†] S.M.A. Corporation Fellow in Biochemistry assigned to Pediatrics.

[1] György, P., and Goldblatt, H., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 492.

During the last 2 years similar investigations have been carried out with the purpose of elucidating the cause of cirrhosis and cancer of the liver in rats brought about by the addition of butter yellow (dimethylaminoazobenzene, dissolved in oil) to a diet consisting of rice and carrots, as first described by Kinosita.[2]

The question arose whether or not the same mechanism that proved effective in the prevention of simple dietary liver injury[1] may also play a rôle in the prevention of changes in the liver which follow the administration of butter yellow.[‡]

Results. 1. In a control group of rats fed basal diet A, consisting of rice, carrots, and dimethylaminoazobenzene in oil (0.6 g per kilo of diet), supplemented with 20 μg daily each of thiamine, riboflavin and pyridoxine and with 100 μg daily of pantothenic acid, cirrhosis, atypical, nodular proliferation of bile ducts and carcinoma[§] of the liver were a regular feature. In different groups the incidence of these changes fluctuated from 80 to 100%. However, only 40% of rats fed the same diet with the addition of 18% casein[‖] showed similar changes. These observations are in close agreement with the results obtained by Kensler and his collaborators[4] just published.

2. Sixty rats were put on basal diet A (rice, carrots, dimethylaminoazobenzene) with the usual supplements of thiamine, riboflavin, pyridoxine and pantothenic acid. In addition sub-group 1 (16 rats) of this experiment received from 10 to 20 mg of choline

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[2] Kinosita, R., *Trans. Soc. Path. Jap.*, 1937, **27**, 665.

[‡] The special series of experiments dealing with the histologic character of these liver changes and with the effect of various vitamin-like substances including biotin, para-aminoazobenzoic acid and dried liver[3] will be reviewed in a separate paper. In this study, as well as in the continuation, as yet incomplete, of the experiments reported here, the staff of the research laboratories of the S.M.A. Corporation has taken and is continuing to take an extensive part.

[3] Nakahara, W., Fujiwara, T., and Mori, K., *Gann.*, 1939, **33**, 57.

[§] The lesion spoken of as carcinoma in these experiments is microscopically identical with that reported by other investigators who have used butter yellow but, so far, in our experience, it has not shown metastases and has not proved transplantable.

[‖] Thiamine, riboflavin, pyridoxine and pantothenic acid have been kindly furnished by Merck & Co., Rahway, New Jersey, and casein by the S.M.A. Corporation, Chagrin Falls, Ohio.

[4] Kensler, C. J., Sugiura, K., Young, N. F., Halter, C. R., and Rhoads, C. P., *Science*, 1941, **93**, 308.

daily, sub-group 2 (12 rats) received from 25 to 50 mg of cystine daily and sub-group 3 (12 rats) received daily from 25 to 50 mg of cystine plus from 10 to 20 mg of choline. Sub-group 4 (20 rats) received no supplement. The results are summarized in Table I.

TABLE I.

Basal diet A		Supplement	Liver changes produced		
			Cirrhosis	Atypical nodular proliferation of bile ducts	Carcinoma
rice carrots thiamine riboflavin pyridoxine pantothenic acid	and dimethylaminoazobenzene (0.6 g per kg diet)	none	+++	+++	++
		cystine	+++	+++	+++
		choline	+++	++	++
		cystine and choline	+	+	0

Incidence of lesions: + slight, ++ moderate, +++ great.

3. Twenty rats kept on diet B, composed of casein 18%, cane sugar 68, melted butter fat 8, salt mixture 4, and cod liver oil 2, and supplemented again with thiamine, riboflavin, pyridoxine (20μg of each daily), with pantothenic acid (100 μg daily) and with the same amount of dimethylaminoazobenzene (0.6 g per kilo of diet) as in basal diet A, were conspicuously free of cancer or of atypical proliferation of bile ducts. Up to 175 days of the experimental period only 2 rats have shown mild patchy necrosis and slight cirrhosis[5] as the only sign of liver injury.

4. Eighty rats were put on diet C, composed of casein 6%, lard 23, cane sugar 15, cornstarch 50, salt mixture 4, cod liver oil 2, supplemented with dimethylaminoazobenzene in the same proportion as in diets A and B. The animals were subdivided into 4 groups. Sub-group 1 received no further supplement, sub-group 2 received 20 mg of choline daily, sub-group 3 received 50 mg of cystine daily and sub-group 4 received 50 mg of cystine plus 20 mg of choline. The 18 rats in sub-group 1 and the 30 rats in sub-group 2 all showed evidence of diffuse and severe hemorrhagic necrosis with or without cirrhosis. In sub-group 3, all 18 rats exhibited marked cirrhosis with or without additional necrosis, whereas in the 14 rats in sub-group 4 only 2 manifested severe cirrhosis, 3 slight cirrhotic or necrotic changes and the remaining 9 were free even of such changes.

In view of all these observations the conclusion appears to be warranted that the lipotropic activity of casein and in particular the combined oral administration of cystine plus choline afford a definite but not regular protection against pathological changes in the liver (necrosis, cirrhosis, atypical nodular proliferation of bile ducts, adenocarcinoma, malignant hepatoma) produced by a diet containing dimethylaminoazobenzene.

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[5] György, P., and Goldblatt, H., *J. Exp. Med.*, 1939, **70**, 185.

Further, it is noteworthy that no malignant changes were seen in rats fed rations containing dimethylaminoazobenzene but no rice (diets B and C). This may be due, apart from the effect of casein (present in diets B and C), either to a special carcinogenic property of rice or to other differences, such as content of butter fat, lard, sugar or corn starch in the rations used.

The experimental period lasted up to 175 days.

The experiments were performed on several hundred rats and are being continued.

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Centripetal Discharges in Dorsal and Ventral Roots Following Stimulation of Muscle by Ventral Root Volleys.

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When a ventral spinal root (L 7 or S 1) of the cat under light dial narcosis (0.5 ml/kilo Ciba) is stimulated, discharges arising in the periphery are conducted centripetally to both dorsal and ventral roots. Afferent activity consequent upon muscle contraction would be expected, but the discharges under consideration are such as to suggest that the mediation of muscle receptors is not involved. Centripetal discharges in ventral roots have been observed following the administration of eserine,[1] or prostigmine.[2] Apparently such discharges have not been observed previously in the non-eserinized preparation.

Dorsal Root Discharges. Record A of the accompanying figure shows the early centripetal activity (spike potentials 1 and 2) recorded from L 7, D. R. following a single shock to L 7, V. R. Similar discharges from the periphery may be recorded if S 1 roots are substituted. Activity of comparable latency, though less intense, may be recorded from S 1 D. R. after an L 7 V. R. shock and vice versa. Spike potentials 1 and 2 follow the stimulus rate at frequencies yielding fused contraction. The later activity of record A suffers immediate conditioning, and is thus, presumably, of different origin.

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[1] Dun, F. T., and Feng, T. P., *Chin. J. Physiol.*, 1940, **15**, 433.

[2] Masland, R. L., and Wigton, R. S., *J. Neurophysiol.*, 1940, **3**, 269.

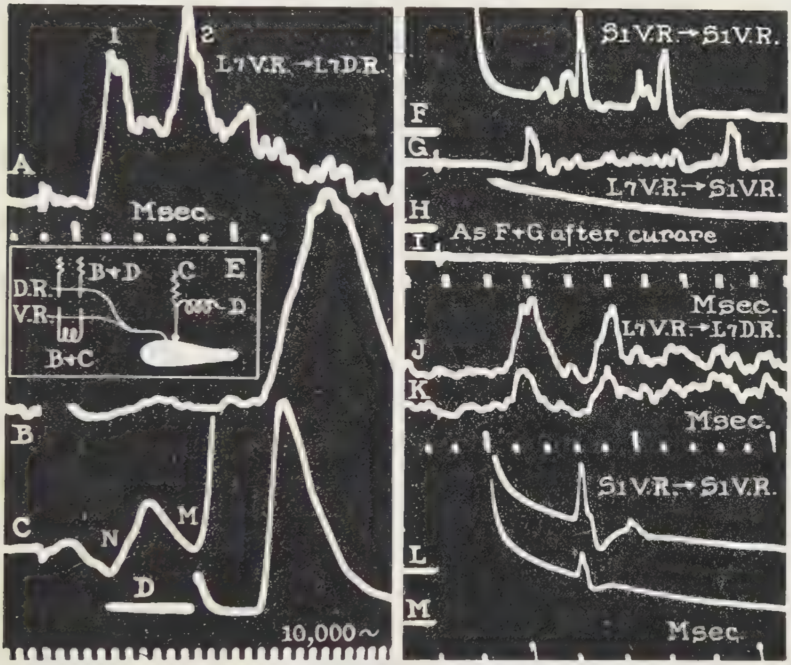

Fig. 1.

Denervation of the distal or proximal muscle groups of the hind leg respectively shows that spike potential 1 arises in the proximal part of the leg, while spike 2 arises in the distal part. Therefore, the succession of spike potentials represents varying conduction times rather than repetitive discharge. Great care must be taken in denervation experiments to identify and avoid discharges due to stimulation at cut nerve ends by nerve action potentials,[3] or by muscle action potentials.[4] The latter is the more serious and enduring artefact.

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[3] Hering, E., *Sitzungsber. d. k. Akad. Wissenschaft. Math.-Naturwissensch. Cl.*, Wien, 1882, **85**, Abt. III, 237; Renshaw, B., and Therman, P. O., *Am. J. Physiol.*, 1941, in press.

[4] Matteucci, C., *C. R. Acad. Sci.*, Paris, 1842, **15**, 797.

Records B, C, and D are recorded from a leg preparation cut down so that only *M. gemellus* superior responds to an S 1 V. R. volley. The details of the root-nerve-muscle preparation and the disposition of electrodes are shown in inset E. The latency of response B in the S 1 D. R. was unchanged from that of the spike potential 1 obtained in the intact leg preparation at the beginning of the experiment. Record C shows the nerve (N) and muscle (M) action potentials recorded at the junctional region. The neuromuscular delay is approximately 0.55 msec., in agreement with the findings of others.[5] Record D shows the conduction time from the muscle to the D. R. leads and is placed so that the stimulus artefact coincides in time with the onset of the muscle action potential (M) of record C. The sum of efferent conduction time, neuromuscular delay, and afferent conduction time approaches the total latency of the return discharge B. This evidence supports the view that the afferent fiber endings are stimulated directly by the activated muscle. The most rapidly conducting fibers are involved.

Ventral Root Discharges. Following a single shock to a ventral root of the intact leg preparation, impulses return from the periphery in the same (F), and adjacent ventral roots (G). Similar discharges occur in stimulated and unstimulated fractions of a split L 7 V. R. Stimulation of L 7 and S 1 roots simultaneously, with leads on one of the roots, results in more than summation of the antidromic discharges; hence some motor fiber endings are stimulated subliminally at the periphery following either of the shocks alone.

Action of Curare. Full curarization abolishes the centripetal discharges in ventral roots, (compare H and I with F and G) and dorsal roots. During partial curarization, reduced centripetal responses occur. Such responses are further reduced if long rest intervals are not allowed between stimulations. This effect parallels the "Wedenski type" inhibition of muscular contraction seen in partial curarization.[6] J and K, obtained at an interval of 1 sec., illustrate the reduced response in the L 7 D. R. and its inhibition. L and M show, in a comparable experiment, the inhibition of ventral root response.

Conclusion. The latency of the discharges under consideration, their parallelism with muscle action in the normal and curarized preparations, and the fact that they occur in fibers other than those carrying the centrifugal motor volley, point to the synchronously active muscle as the causal agent. The suggestion may be advanced that the action potential of the excited muscle is capable of stimulating intramuscular motor and sensory nerve endings.

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[5] Lorente de Nó, R., *Am. J. Physiol.*, 1935, **111**, 272; Eccles, J. C., and O'Connor, W. J., *J. Physiol.*, 1939, **97**, 44.

[6] Hofmann, F. B., *Pflüger's Arch.*, 1903, **93**, 186; Bremer, F., and Titeca, J., *Arch. Internat. Physiol.*, 1935, **42**, 223; Rosenblueth, A., and Morison, R. S., *Am. J. Physiol.*, 1937, **119**, 236.

13033

Bactericidal Effect of Ultraviolet Rays on Non-Spore Forming Bacteria and Mold Spores.

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Fellers[1] and Garret and Arnold[2] reported effective sterilization of drinking glasses by ultraviolet light produced by the "Sterilamp". Owing to the ease with which such objects can be exposed, this means of sterilization has many advantages. The "Sterilamp" was employed in this investigation as the source of ultraviolet light. In one series of experiments exposures of drinking glasses were made in the Steril-Ray Cabinet, in which 4 Sterilamps were used. For the remainder of the experiments, results of which are reported in this investigation, a laboratory cabinet, fitted with 2 Sterilamps, was used. The inside measurements of this cabinet were 14 x 14 x 10.5 inches and it was lined with polished aluminum foil. The shelf was made of one-half inch mesh screen and placed about 8 inches from the lower surface of the Sterilamps.

The first experiments were carried out with suspensions of bacteria and mold-spores in Petri dishes. Films of nutrient broth cultures were made on the bottoms of sterile Petri dishes as suggested by Ross.[3] Agar medium in Petri dishes was also sprayed with an atomizer. Another method of inoculation was to rinse the containers with a suitable suspension of the bacteria. In order to study the lethal effect of ultraviolet light on non-sporulating bacteria and on mold-spores, one ml portions of the suspensions were placed in sterile Petri dishes, exposed definite times to the light, and finally melted agar was added to the exposed suspensions. The most satisfactory method for inoculation consisted of rinsing the drinking glasses with the suspension of bacteria and draining for about one minute after which the receptacles were shaken to remove the greater part of the liquid. Glasses were kept covered with parts of sterile Petri dishes except when being exposed to the ultraviolet light.

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[1] Fellers, R. V., *The American City*, August, 1938.

[2] Garret, O. F., and Arnold, R. B., *Milk Plant Monthly*, August, 1938.

[3] Ross, Virginia L., M.S. Thesis, University of Michigan, 1938.

The following non-sporulating bacteria were used in the preliminary work: *Escherichia coli* (Frank strain), *Escherichia coli* (American Type Culture Collection No. 4348), *Staphylococcus aureus,* and *Serratia marcescens.* The results of a semi-quantitative study using various dilutions of *Escherichia coli* (Frank strain), which were exposed in the laboratory cabinet, are given in Table I. These results indicate clearly the relation of the initial concentration to the time required to kill all of the organisms. Too little importance has been given to this factor. However, if the initial numbers are made comparable, then the relative resistance of different bacteria and mold-spores to exposure may be compared. In this way it was found that order of decreasing resistance to ultraviolet light for the bacteria studied was: *Staphylococcus aureus, Escherichia coli* (Frank strain), *Escherichia coli* (A. T. C. No. 4348), and *Serratia marcescens.*

TABLE I.
Effect of Concentration of *Escherichia coli* (Frank strain) to Exposure to Ultraviolet Light. (Laboratory cabinet.)

Approx. initial conc. of *Escherichia coli*	Growth after exposures of: (min.)						
	0	0.5	1	2	3	4	5
12×10^6	++++	+++	+	+	+	30	3*
12×10^4	+++	++	0	1	0	0*	4*
12×10^3	++	40	1	0	0	0*	30
12×10^2	+	0	0	0	0	0	1

*Colonies unlike those of *Escherichia coli* were present.

Exposures of mold-spores in suspensions showed the same general results as were obtained with the bacteria tested. Four different molds were used, namely, *Aspergillus glaucus, Aspergillus niger,* a Mucor, and a Penicillium. Some difficulty was experienced at first in the preparations of suspensions free from clumps of spores and from vegetative cells; however, the following procedure gave excellent results. Dextrose-agar-slant culture (8 to 16 days old) was flooded with salt solution, shaken, and, in most cases, the growth gently rubbed with the tip of a sterile wire. This suspension was poured into a sterile tube and the process repeated using, in all, 10 ml of salt solution. The suspension was mixed thoroughly by shaking vigorously and then filtered through 12 thicknesses of sterile cheesecloth. It was found necessary to wet the filter with about 5 ml of salt solution before pouring the spore-suspension on it in order not to lose most of the suspension on the filter. The suspension was refiltered through the same filter (2 or 3 times) until examination with the microscope showed it to be free from clumps and vegetative cells. After counting the spores (Neubauer chamber) the suspension was diluted sufficiently to give readily countable plates on the controls. One ml portions were exposed in each Petri dish. The results obtained in one series of exposures are given in Table II. It will be noticed that the effect of irradiation was either partial or complete depending upon the initial concentration of spores. As the concentration decreased, the effectiveness of the irradiation as a sterilizing agent increased.

TABLE II.

Effect of Ultraviolet Light upon Mold-Spores in Suspensions. (Laboratory cabinet used)

Mold used	Growth estimates after 48-hr incubation, room temperature. Exposure time in min.								
	0	1	2	3	4	5	6	7	8
Aspergillus glaucus	++++								
Dilution 1:100	(10,000)*	+++	++	150	200	200	100	100	
1:10,000	100	90	5	3	2	3	0	0	
Aspergillus niger	++++								
Dilution 1:100	(40,000)*	+++	+++	++	+	200	200	130	
1:10,000	400	130	15	2	0	1	1	1	
Mucor									
Dilution 1:100	++++	+++	30	10	8	3	4	5	
1:10,000	+++	+++	30	2	4	1	2	0	
Penicillium	++++								
Dilution 1:10	(330,000)*	+++	++	+	+	300		300	200
1:1,000	(3,300)*	40	5	6	1	1		0	0
1:10,000	330	4	0	0	0	0		0	0

*Figures obtained from count at 1:10,000 dilution.

In another series of exposures it was found that when the spores had germinated before exposure, a much greater resistance was evident. Examination with the microscope of the inoculated agar surface after 18 hours of incubation at room temperature revealed no mycelia in the cases of *Aspergillus glaucus* and the Penicillium; however, in the experiment with *Aspergillus niger* and the Mucor mycelia were present. From these observations the penetration of the hyphæ into the agar seems to have afforded a considerable degree of protection.

Experiments were also carried out to determine the lethal effect of ultraviolet rays on mold-spores present on paper board. Paper and paper board have been suggested as the cause of mold spoilage of certain foods packaged in these materials. Increasing use of these materials has made it desirable to determine whether the contaminating microörganisms can be eliminated by irradiation with ultraviolet rays. Strips of cardboard (12 x 65 mm), such as is used for food containers, were sterilized in an autoclave. Each strip was inoculated separately by immersion for approximately 2 minutes in a suspension of mold-spores. They were then removed with sterile forceps, allowed to drain, and kept a short time in a sterile tube. The suspension of spores, practically free of clumps and vegetative cells, was prepared as previously described. By the method of inoculation which was employed it seems probable that many spores were introduced into the fibers.

The strips were exposed in pairs by inserting the ends of each strip into the wire mesh of the shelf in the laboratory cabinet. By this procedure the actual contact between strip and shelf was minimal. In addition, indirect exposure of the under side of the strip was more satisfactorily obtained. After a given exposure the strip was removed and placed in about 5 ml of melted dextrose-agar which had just been poured into a sterile Petri dish; following this another 5 ml of melted dextrose-agar was evenly distributed over the strip. As a result of this procedure, the strip was completely coated with agar. Incubation at room temperature for 48 hours was found to be satisfactory for making estimates of colony numbers. Check estimates made after 72 hours of incubation were difficult to evaluate. However, the results appeared to agree with findings obtained after 48 hours' growth. Table III gives the results of one such series of exposures and it shows clearly the ineffectiveness of ultraviolet light. Incomplete sterilization resulted in the case of *Aspergillus niger*. The fact that the under side of the strips frequently showed many more colonies than did the upper side indicated that the indirect exposure was much less effective than the direct one.

TABLE III.
Effect of Sterilamps on Mold-spores on Cardboard Exposed in the Laboratory Cabinet.

	Estimates of spores per strip after exposure of : (min.)															
Incubation of (hr)	0		0.5		1		2		3		4		5		10	
Mold used	48	72	48	72	48	72	48	72	48	72	48	72	48	72	48	72
Aspergillus glaucus	55	85	43*	50	31*	34	16*	26*	6*	23*	7*	14*	3*	3*	1*	1*
Aspergillus niger	40	47	28	27	13	17	12*	15*	3*	15*	5*	11*	6*	13*	6*	7*
Mucor	8	8	9	9	3	3	0	0	0	0	0	0	0	0	0	0
Penicillium	80	**	21*	27*	26*	36*	2*	10*	3*	5*	3*	4*	4*	6*	0	1*

*Most of colonies on under side of strip.
**Too crowded to enumerate.

Summary. The results secured in these investigations show that the initial number of cells influences survival of microörganisms exposed to ultraviolet light. Dilute suspensions are destroyed more quickly than heavy ones. The order of decreasing resistance for the species used was: *Staphylococcus aureus, Escherichia coli* (Frank strain), *Escherichia coli* (American Type Collection No. 4348), and *Serratia marcescens.* For the mold-spores studied the order of decreasing resistance was: *Aspergillus glaucus, Aspergillus niger,* Penicillium, and Mucor.

Cardboard strips, which had been inoculated by immersion in a suspension of mold-spores, were not as thoroughly sterilized as were the suspensions themselves, when exposed to ultraviolet light in Petri dishes. On many of the strips it was possible to determine that the colonies originated from the under side of the strip more frequently than from the upper side. This was interpreted as indicating that indirect irradiation is much less effective than direct irradiation.

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Bactericidal Effect of Ultraviolet Rays on Microorganisms on Restaurant Glass-Ware.

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To study the bactericidal effect of irradiation with the Sterilamp on drinking glasses which had been used at soda fountains, it was necessary to select or devise a method of sampling the bacteriological contamination of the glasses. The multiple glass test proposed by the American Public Health Association[1] was used whenever a series of glasses from a soda fountain was examined. Other procedures were followed including the method used by Speck and Black.[2] In addition, a supplementary method was devised which permitted examination of the entire inner surface of the glass as well as the rims and proved to be simpler than that of Speck and Black. About 5 ml of a 25 ml water blank were poured into the drinking glass to be sampled and the entire inner surface of the glass was wetted and rubbed by means of a sterile cotton swab. The swab was then broken off into the rest of the 25 ml water blank and shaken vigorously until the cotton had become well disentangled. The cotton swab and the remainder of the water blank were then carefully transferred back to the drinking glass. The contents of the glass were mixed by a few rapid rotations and one ml portions were plated on agar.

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[1] Am. Pub. Health Assn., Yearbook, 1936-1937, p. 48.

[2] Speck, M. L., and Black, L. A., *Food Research,* 1937, **2**, 559.

During this investigation an opportunity was presented to make some tests with a Sterile-Ray Cabinet. A series of unirradiated dry drinking glasses, a series of irradiated dry drinking glasses, a series of unirradiated wet (but clean) drinking glasses and a series of irradiated (but clean) drinking glasses were examined bacteriologically by the American Public Health Association procedure and by the procedure of Speck and Black. The exposure time of 130 seconds was controlled by a timing device on the cabinet. The plate counts on nutrient agar were made after a 24-hour and a 60-hour incubation period at 37°C. The results appear in Table I. It will be noted

TABLE I.
Exposure of Drinking Glasses for 130 Seconds in the Steril-Ray Cabinet.

	Estimates of colonies per glass after being incubated for: 24 hr	60 hr
A.P.H.A. proposed procedure used on:		
10 unexposed glasses	3.5	15.5
10 exposed glasses	0.3	9.3

	Estimates of colonies on rims from plate counts after incubation of: (Inside rim) 24 hr	(Inside rim) 60 hr	(Outside rim) 24 hr	(Outside rim) 60 hr
Procedure of Speck and Black (1937) used on:				
Dry unexposed glass	25	25	100	500
” ” ”	50	125	0	75
” ” ”	0	25	25	25
” ” ”	0	75	0	0
Dry exposed glass	0	0	0	0
” ” ”	0	0	0	0
” ” ”	0	25	0	0
” ” ”	0	0	0	25
Wet unexposed glass	2,950	*	75	275
” ” ”	27,500	*	75	275
” ” ”	550	1,325	175	450
” ” ”	1,150	2,550	75	250
Wet exposed glass	0	25	25	50
” ” ”	0	0	0	25
” ” ”	50	100	0	25
” ” ”	0	50	150	350

*Not possible to count colonies.

TABLE II.
Effect of the Sterilamps on Drinking Glasses Rinsed with Suspensions of *Escherichia coli* (Frank Strain).

	Estimates of colonies on the inside surface of the glass after being exposed for: (min.)					
	0	1	2	3	4	5
Glass A	7500	1300	0	0	0	0
Glass B	(7500)*	325	350	75	0	25

*Count taken equal to the first because it was too difficult to estimate.

TABLE III.
Effect of Sterilamps on Drinking Glasses Exposed in the Laboratory Cabinet.

Estimates of colonies on the inside surface of the glass after the exposure indicated:		
5 unexposed glasses	5 glasses exposed for 2 min.	5 glasses exposed for 4 min.
250	0	75
600	0	50
200	0	50
1000	2120	25
550	625	25
Avg 520	Avg 549	Avg 45

that, while the exposed and unexposed dry glasses show only small differences in counts, the opposite is true for wet (but clean) glasses.

In another series of experiments, sterile drinking glasses were rinsed with a suspension of approximately 120,000 bacteria per milliliter. The glasses were irradiated in pairs for definite time intervals in the laboratory cabinet. Table II shows clearly the marked lethal effect of the radiation on *Escherichia coli* (Frank strain).

In a still different type of experiment, 15 clean and dried drinking glasses were placed in individual sterile paper bags at the soda fountain and then transported to the laboratory for sampling and exposure. Five of the test glasses were sampled as untreated controls; 5 were exposed in the laboratory cabinet for 2 minutes and then sampled; the remaining 5 were exposed for 4 minutes before sampling. Sampling was done with 25 ml water blanks and single swabs as previously described in this article. The counts were made after 48 hours incubation at 37°C and 96 hours at room temperature. The results in Table III show a reduction of about 90% for a 4-minute exposure, but no reduction in the average count for a 2-minute exposure. It should be indicated, however, that in groups of glasses exposed for 2 minutes, three were apparently devoid of any living bacteria.

Summary. A sampling procedure, which was developed in order to examine more completely the interior of a drinking glass, proved quite satisfactory and it was used extensively in this investigation.

The Steril-Ray Cabinet, employing 4 Sterilamps, was tested while in actual operation at a soda fountain. Dry clean glasses showed but slight bacteriological improvement as the result of being exposed in this cabinet. It should be noted that these were already of low bacterial content without being exposed. Wet clean glasses showed a very marked improvement as the result of being exposed. However complete sterilization did not result in every case.

The experimental evidence obtained in this investigation indicates that almost sterile surfaces are produced when clean drinking glasses are irradiated with the ultraviolet light produced by the Sterilamp.

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Determination of Hydrogen Sulfide in Stomach Contents of Experimental Animals.

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The problem of determining the sulfide content of solutions containing reducible or interfering substances presents difficulties. Since hydrogen sulfide is unstable and easily oxidized considerable trouble may be expected in recoving it from solution. Aeration with air, carbon dioxide, or hydrogen as well as distillation procedures have been employed[1, 2, 4, 5] but the completeness of the removal is open to question.

Recently in connection with another investigation it was necessary to determine the hydrogen sulfide concentration in the stomach contents. Due to the nature of the material it was evident that methods based on evolution procedures were the only approach to the problem.

Preliminary experiments with various evolution procedures on pure hydrogen sulfide solutions (1-10 mg per liter) consistently

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[1] Benade, W., *Mitt. Lab. preuss. geol. Landesenst*, 1933, **19**, 3; Dept. Sci. Ind. Research, *Water Pollution Research*, Summary, current lit. **7**, 318.

[2] Buswell, *J. Am. Chem. Soc.*, 1925, **47**, 1381.

[3] Gibbs and Clardy, *Ind. and Eng. Chem. Anal. Ed.*, 1940, **12**.

[4] Marzahn, W., *Hyg. Rundschau*, 1919, **29**, 557; *Exp. Stat. Record*, **42**, 207.

[5] Rudolfs and Baumgartner, *Ind. and Eng. Chem.*, 1932, **24**, 1152.

gave results which varied from 50-90% of theory. Furthermore, the sulfide ion concentration rapidly diminished on standing. Other investigators[2] have reported similar observations, but the possibility of errors of this magnitude has not been generally recognized.

In the determination of the sulfide content of metals and alloys, evolution procedures give excellent results.[6] This is probably due to the presence of nascent hydrogen which reduces any free, sulfite, or thiosulfate sulfur to sulfide ion, thus preventing any oxidation or decomposition of hydrogen sulfide.[7] This suggests that proper reducing conditions in sulfide solutions may permit quantitative removal of hydrogen sulfide.

On the basis of these considerations the following method was developed which may be useful to those confronted with a similar problem.

Apparatus and Procedure. 1. 0.01 N sodium thiosulfate solution containing 1 volume % of amyl alcohol. 2. 12 N hydrochloric acid. 3. Cadmium chloride solution: 10 g of cadmium chloride dissolved in 400 ml of water and diluted with 600 ml of 28% ammonium hydroxide. 4. Starch solution: 2 g of soluble starch dissolved in 250 ml of boiling water and filtered. 5. Caprylic alcohol. 6. 0.01 N iodine solution, 1 ml = 6.8 mg per liter of hydrogen sulfide per 25 ml sample.

In an apparatus similar to one described by Gibbs and Clardy[3] an 8-in. test tube served as the reaction vessel and a 6-in. test tube as the receiver. The thistle tube was equipped with a segment of rubber tubing and a screw clamp to permit the closing of the inlet tube.

The reaction vessel was charged with a 25 ml sample of material, 2-3 g of metallic zinc (S-free) and a drop or two of caprylic alcohol. The absorption tube containing 1 ml of cadmium chloride diluted to 15 ml was then placed in the system. Twenty-five ml of 12 N HCl were added to the reaction vessel, and the inlet tube was closed by means of the screw clamp. To hasten the evolution of hydrogen sulfide, the reaction vessel was warmed at the beginning. After the evolution of hydrogen was complete, the reaction vessel was brought to boiling for a minute or two, the receiver contents acidified and treated with an excess (1-2 ml) of standard iodine solution; back titration was with standard thiosulfate solution measured from a microburet.

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[6] A. S. T. M., *Methods of Chemical Analysis of Metals,* 1939, E **30-39**, 23.

[7] Schere and Sweet, *Ind. and Eng. Chem. Anal. Ed.,* 1932, 103.

Results and Discussion. The results of several typical experiments (Table I) show that the quantitative removal of hydrogen sulfide from stomach contents can be attained by the use of nascent hydrogen as the aerating agent. When air was used instead of nascent hydrogen consistent but slightly lower results were obtained. The recovery of added amounts of hydrogen sulfide was always incomplete due probably to oxidation.

When nascent hydrogen is used for aeration of the hydrogen sulfide, other reduced forms of inorganic sulfur must be absent.[7] Pure solutions of cysteine and cystine evolved small amounts of hydrogen sulfide when treated with nascent hydrogen. When cystine, cysteine or methionine was added to stomach contents no additional hydrogen sulfide was obtained (Table I).

TABLE I.
Determination of Hydrogen Sulfide by Aeration with Nascent Hydrogen.

				Total H_2S mg/liter	
	Stomach contents mg H_2S/liter	Mg added/liter		Found	Calc.
I. Recovery experiments	3.7	8.6	H_2S	11.9	12.3
	4.4	8.0	”	12.6	12.4
	5.1	7.8	”	12.9	12.9
II. Effect of organic sulfur on recovery of H_2S		*4,000	cystine	0.6-1.8	
		*4,000	cysteine	0.6-3.0	
	2.7	4,000	cystine	2.7	
	2.7	4,000	cysteine	2.7	
	2.7	4,000	methionine	2.7	
III. Typical analyses of a given sample	3.9				
	3.8				
	3.8				
		Standard H_2S solution (made slightly alkaline) Mg H_2S/L.			
IV. Stability of sulfide solutions	3.0	6.7			
	2.6	1 day later 5.0			
	2.7	2 ” ” 3.3			
	2.1	3 ” ” 2.2			

*With pure solutions the results varied through limits indicated.

The stability of sulfide ion is slightly greater in stomach contents than in pure sulfide solutions; perhaps this might be expected from the nature of materials present.

Summary. A simple method is described for determining hydrogen sulfide in the stomach contents of experimental animals.

13036

Activity of Toad Lymph Hearts.

V. G. Foglia and E. Braun-Menendez. (Introduced by C. D. Leake.)

From the Instituto de Fisiologia, Facultad de Medicina, Buenos Aires.

The pulsations of the posterior lymph hearts of the toad, *Bufo arenarum* Hensel, have been recorded[1] by the optical method in the following way: a small funnel was applied over the pulsating zone and connected by rubber tubing to a Frank's capsule covered with a very delicate membrane such as that used for heart sound recording.[2] The records obtained were of good amplitude especially if the skin covering the lymph hearts was previously removed. The characteristic irregularity of the recorded pulsations was found to be due almost exclusively to variations in the duration of diastole. Bi- and tri-geminal rhythms were sometimes observed.

A very remarkable fact, which we have verified in more than 150 observations, is that *the 2 posterior (left and right) lymph hearts have always the same frequency per unit of time.* This occurs in spite of the arrhythmia of individual hearts, the absolute asynchronism between left and right hearts, and the varying frequencies observed at any temperature between 0° to 40°C.

The variation of pressure within the lymph hearts has been also recorded by introducing a fine needle into the cavity and connecting it with the recording capsule as described. The curve of intracardiac lymph pressure shows a rise in pressure together with the beginning of contraction. The rise is first slow and then more rapid; the decline in pressure is not so steep as the rise. It is difficult to ascertain in which point of the curve systole ends. Measurements show that the average period between the first rise in the pressure curve and its return to the base line, lasts from 0.46 to 0.55 sec. (Fig. 1.)

In about 150 normal toads kept in a humid atmosphere, the frequency of the lymph hearts was determined at different temperatures from 0° to 37°C. The frequency increases with the temperature. We noted an average of 15 beats per minute at 0° and 123 beats per minute at 37°C. At room temperature (19°C) the mean

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[1] Foglia, V. G., and Braun-Menendez, E., *Rev. Soc. Argent. Biol.*, 1939, **15**, 178; *Compt. rend. soc. biol.*, 1940, **133**, 328.

[2] Orias, O., and Braun-Menendez, E., *The Heart Sounds in Normal and Pathological Conditions,* Oxford University Press, London, 1939, p. 29.

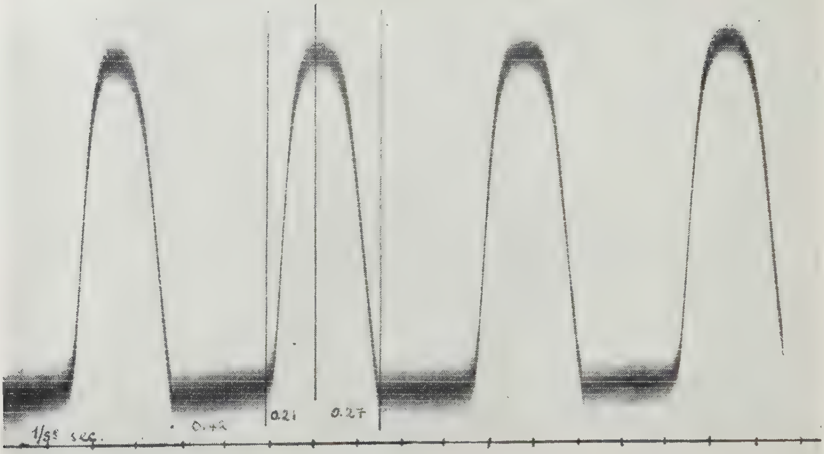

FIG. 1.
Optical record of the variations of intracardial pressure of the posterior lymph heart. Time in fifth of second.

frequency was observed to be 50 beats per minute. The increase in frequency follows the Van't Hoff law between 0° and 20°C.

The frequency of the lymph hearts was found to be less in hypophysectomized and in adrenalectomized toads than in the normal controls (Table I).

TABLE I.
Effects of Hypophysectomy and Adrenalectomy on Frequency of Toad Lymph Heart Contraction.

	No. of toads	Mean frequency of post. lymph hearts per min. at 20°C	
		Right	Left
Hypophysectomized, 8 days	10	40.6	40.4
Controls, ” ”	8	48	50
Adrenalectomized, 2 ”	8	45	45
Controls, ” ”	4	54	53

The gross effects of certain representative cardiac drugs on the frequency and amplitude of contractions of the posterior lymph hearts was studied after injection of 0.5% to 2% solutions into the dorsal lymph sac. Some observations were made following local application after removing the skin covering the posterior lymph hearts.[3]

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[3] Foglia, V. G., and Braun-Menendez, E., *Rev. Soc. Argent. Biol.*, 1939, **15**, 235; *Compt. rend. soc. biol.*, 1940, **133**, 331.

We noted that curare (1%), erythrine (1%), acetylcholine (2%), and nicotine (0.5%) applied locally or injected into the lymph sac produced an immediate stoppage of the contractions of the lymph hearts. Adrenaline (1%), though not constantly, had the same effect. Strychnine (1%) and veratrine (1%) also stopped the heart beats but only after the general symptoms of intoxication had appeared. Eserine (0.5%), veratrine (1%) and ouabain (1%) applied locally caused slight diminution in the amplitude of the beats. Pilocarpine (1%), atropine (1%), pitressin and pitocin did not affect the activity of the posterior lymph hearts.

13037 P

Ovulatory Response of *Rana pipiens* to Mammalian Gonadotropic Factors and Sex Hormones.

William B. Langan. (Introduced by L. G. Barth.)

From the Biological Laboratories of Columbia and Fordham Universities, New York.

The complete refractoriness of the common frog, *Rana pipiens,* to mammalian gonadotropic factors has attracted considerable attention, especially from point of view of comparative endocrinology and when considering such concepts as species gonadotropic specificity.[1] On the other hand, stimulation of the reproductive tracts of immature mice has been brought about by large doses of fresh anterior pituitary from *Rana pipiens.*[2] For further investigation of this problem the following preliminary experiment was undertaken.

Twenty-nine adult female *Rana pipiens* received abdominal injections, in varying concentrations, of (1) gonadotropic factors of mammalian anterior pituitary, (2) gonadotropic factors of pregnant mare's serum (gonadogen), (3) α-estradiol dipropionate (di-ovocylin), (4) progesterone (lutocylin), and (5) testosterone propionate (oreton). Injections of distilled water, sesame oil, and homoplastic pituitaries served as controls.

Approximately 72 hours after treatment an attempt to strip eggs was performed, immediately followed by laparotomy. Portions of oviducts and ovaries were preserved for histological study while the pituitaries were prepared for cytological study. The ovulatory response was recorded as the relative number of eggs in the oviducts. In cases of negative response to stripping, a pituitary from a normal female frog was injected, which in all cases resulted in complete ovulatory response thus demonstrating that these specimens were capable of responding.

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[1] Creaser and Gorbman, *Quart. Rev. Biol.*, 1939, **14**, 311.

[2] Adams, E. A., and Granger, B., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 585.

TABLE I.

Ovulatory Response of *Rana pipiens* to Mammalian Gonadotropic Factors and Sex Hormones. Temperature 15°C.

No. of Frogs	Substance injected	cc	Dosage	Ovulatory response %
2	*Gonadotropic factors of mammalian anterior pituitary	1	300 units (Collip)/cc	—
2	Gonadotropic factors of mammalian anterior pituitary	2	” ” ” ”	—
3	†Gonadotropic factors of pregnant mares serum	1	10 ” (Cartland-Nelson)/cc	—
2	Gonadotropic factors of pregnant mares serum	2	10 units (Cartland-Nelson)/cc	—
3	‡α-estradiol dipropionate	2	0.2 mg/cc	—
2	” ”	2	” ”	—
1	§Progesterone	2	1 ”	+ 5
2	”	1	1 ”	+ 50
1	”	.4	1 ”	+ 10
1	”	.4	1 ”	+ 5
1	‖Testosterone propionate	.5	25 ”	+ 100
1	” ”	.5	25 ”	+ 25
1	” ”	.4	25 ”	+ 25
1	” ”	.4	25 ”	—
1	” ”	.3	25 ”	+ 10
1	” ”	.3	25 ”	+ 10
1	” ”	.2	25 ”	+ 100
1	” ”	.2	25 ”	+ 10
1	” ”	.1	25 ”	+ 25
1	” ”	.1	25 ”	—
2	Sesame oil	.5		—
2	Distilled water	1		—
2	*Rana pipiens* pituitary	1	pituitary ♀	+ 100

*Courtesy of Armour Laboratories.
†“Gonadogen,” courtesy of Upjohn Co.
‡“Di-ovocylin,” courtesy of Ciba Pharmaceutical Products.
§“Lutocylin,” courtesy of Ciba Pharmaceutical Products.
‖“Oreton,” courtesy of Schering Corporation.

The substances injected, dosage and ovulatory response are recorded in Table I. This shows that 4 frogs treated with varying doses of mammalian anterior pituitary gonadotropic factors, were completely refractory. Also 5 frogs receiving varying doses of gonadotropic factors of pregnant mares serum, were completely refractory. Likewise, 5 frogs treated with varying doses of α-estradiol dipropionate, gave no response. However, all 5 frogs injected with varying doses of progesterone gave positive ovulatory responses, ranging from 5 to 50%. Of 10 frogs treated with varying doses of testosterone propionate, 8 gave positive responses ranging from 5 to 100%, while 2 failed to respond. The controls of distilled water and sesame oil were completely negative, while the pituitary controls were completely positive.

13038

Sex Hormones and Lymphomatosis in Fowls.*

David Marine and S. H. Rosen.
From the Laboratory Division, Montefiore Hospital, New York City.

We have previously reported[1] upon the occurrence of lymphomatosis in 26 of 52 white leghorn capons and its absence in 66 slips of the same group of 118 birds. However, only 19 of these slips were older, when sacrificed, than the youngest bird (256 days) that developed the disease. There were 10 cases of diffuse hepato-renal lymphomatosis (big livered series), 7 of lymphosarcoma, 8 of lymphoma of bursa of Fabricius and 1 leukemia. The significant finding is that we have not seen lymphomatosis develop under our conditions in a bird with spontaneous comb growth. These observations suggested that the presence of a functionally active fragment of testis may have inhibited the development of the disease—possibly because of the production of sex hormones.

The relation of sex to the incidence of lymphomatosis has not been studied and for obvious reasons. Oakley[2] is the only author to refer to it but gives no data—merely stating that he has the impression it is more common in females and in the breeds kept for egg laying. Data on the sex incidence of leukosis in mice also are lacking.

The present experiment includes 41 white leghorn male castrates of the same age, divided into 3 groups as follows: (1) 12 birds, each receiving 1 mg estradiol dipropionate weekly, (2) 12, each receiving testosterone dipropionate in variable amounts twice weekly,

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* We are indebted to the Ciba Pharmaceutical Products, Inc., for generous supplies of Di-ovocylin and testosterone dipropionate.

[1] Marine, D., and Rosen, S. H., *Am. J. Cancer*, 1940, **39**, 315.

[2] Oakley, C. L., *Proc. Roy. Soc. Med.*, 1935, **28**, 999.

and (3) 17 controls. Injections of these hormones were started on June 18, 1940, and they are still being given to the survivors. To date (Jan. 15) 4, or 33⅓%, of the androgen group, 7, or 58%, of the estrogen group, and 4, or 23%, of the control group have died of lymphomatosis. The youngest (receiving estradiol dipropionate) was 201 days old and the oldest to date, 319 days.

Routine liver biopsies have shown that this organ may be transformed from the normal (about 40 g) reddish brown color to a huge (300 g) reddish gray organ in 15 days. In only one of the 15 cases (a bird receiving testosterone dipropionate) was a fragment of testis found at autopsy—confirming our observation in the first series.

It is probable that some of the surviving birds in the androgen and estrogen groups are slips but masked by the sex hormones administered (one of the estrogen group frequently crows). Anatomically the 15 cases may be grouped as (1) diffuse hepato-renal (big liver disease) type—8 cases, (2) lymphosarcoma—4 cases, (3) lymphoma of the bursa of Fabricius—2 cases, and (4) neurolymphomatosis—1 case.

The polymorphism of lymphomatosis has always attracted the attention of investigators. This polymorphism exists in all 3 major groups (myeloid, erythro and lymphoid) of leukosis as defined by Ellermann.[3]

Routine blood smears made at monthly intervals or oftener have been constantly negative for leukemia as Ellermann and others had pointed out. The incidence of lymphomatosis in the androgen-treated group may not be significant since the doses of testosterone dipropionate during the first 4 months of the experiment varied from excessive to a submaintenance (of the comb) level. The occurrence of lymphomatosis in 58% of the estrogen group as opposed to 23.5% of the control group suggests that the induced sex hormone imbalance may have been a factor in stimulating or activating the agent responsible for overgrowth of lymphoblastic tissue in this group. The doses of hormone used were insufficient to induce changes in the blood calcium and lipoids and, therefore, may not have induced a sufficient hormone imbalance in some of the birds to activate the latent responsible agent.

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[3] Ellermann, V., *Folia Haematol.*, 1923, **29**, 203.

13039 P

Field Study of Ground Squirrel (*Citellus beecheyi*) in Relation to Sylvatic Plague.*

F. C. Evans and R. Holdenried. (Introduced by K. F. Meyer.)

From the George Williams Hooper Foundation for Medical Research, University of California, San Francisco.

This investigation was carried on in 1940 at the Calaveras Reservoir, Alameda and Santa Clara Counties, California. (The area had already been designated as a sylvatic plague focus by the survey made in 1933.) This study involved the live-trapping and marking of 433 individual squirrels. Approximately 100 additional animals were obtained from the area for plague transmission experiments at the Hooper Foundation.

Young squirrels were first seen on May 20th and by the middle of June nearly all the young of the year had appeared. There was no evidence to suggest more than one litter per mother per year in this area, and the period from the beginning of breeding until the appearance of the young above ground was not much more than 2 months.

The young squirrels averaged about 100 g when they first appeared, but they grew rapidly and averaged more than 300 g by the middle of July. The average weight of adult females was 600 g and of adult males was 700 g.

Of the 433 marked squirrels, there were recorded movements for 82. The greatest distance moved was about 1300 yards, while the average of these 82 records was approximately 350 yards. The records include movements by 10% of the total number of marked adults and 22% of the total marked young. This suggests a greater tendency on the part of the young squirrels to move. That these movements are exploratory rather than migratory in nature is indicated by the fact that 45% of all squirrels included in the movement records returned to the area in which they were first captured.

The number of squirrels observed regularly for at least 3 months totalled 135. Of these 90% were never caught outside ranges of 300 yards in diameter and 70% remained within areas of not more than 100 yards in diameter. This significant fact strongly suggests that in the Calaveras Reservoir area the ground squirrel is a sedentary and not a migratory species.

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* This study was aided by a grant from the Rosenberg Foundation.

Fig. 1 illustrates the relative size and composition of the ground squirrel population in 6 consecutive months of the study. Greatest numbers were present in June, when the young squirrels first appeared. There was a steady decline in numbers from June to October. In October over 80% of the population consisted of young of the year.

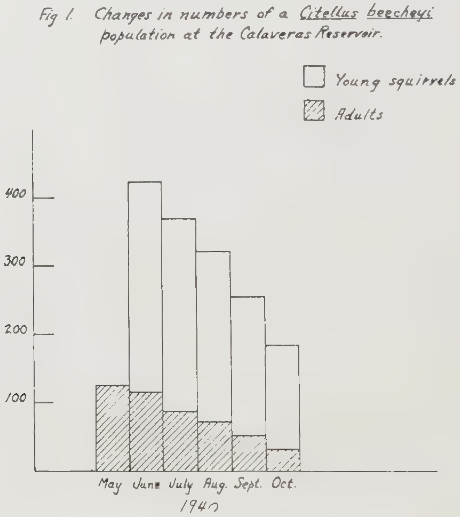

FIG. 1.

The apparently sedentary nature of the ground squirrel population and the fluctuations in numbers of individuals is of importance in determining the rôle of this species in the maintenance and spread of sylvatic plague. It is suggested (1) that extensive migrations of rodent hosts are not necessary for the spread of sylvatic plague, (2) that sylvatic plague in this country probably did not spread by such migrations, and (3) that the occurrence of plague in much of the western United States is due to a process of contact between individuals in a continuous population composed of many host species.

13040 P

Transmission Studies of Sylvatic Plague.*

C. M. Wheeler and J. R. Douglas. (Introduced by K. F. Meyer.)

From the George Williams Hooper Foundation for Medical Research, University of California, San Francisco, California.

The evaluation of the vector efficiency of a species of arthropod incriminated in the transmission of an infectious agent must necessarily take into consideration the following factors: (1) The *infection potential*, (2) the *vector potential*, and (3) the *transmission potential*. The *infection potential* is based upon the percentage of a given species in which the infection becomes established or the disposition of this species to accept the invasion of the organism of the infection. The *vector potential* is based upon the percentage of *infected* individuals which become *infective*. The *transmission potential* is the mean number of transmissions effected by a group of *infective* individuals. The product of these factors will represent the number of transmissions effected by a given number of any species or the *vector efficiency*.

To determine if a given species of flea was capable of transmitting *Pasteurella pestis* (Yersin) mass feeding experiments were first conducted. When transmission ability was established in a species this species was then tested to determine the vector efficiency by daily individual feedings. Mass tests were conducted with *Diamanus montanus* (Baker) and *Hoplopsyllus anomalus* (Baker), predominant species of the California ground squirrel, *Citellus beecheyi* (Richardson), *Nosopsyllus fasciatus* (Bosc) and *Xenopsylla cheopis* Rothschild, the principal fleas of rats, *Rattus* spp., *Malareus telchinum* Rothschild of the field mouse, *Microtus californicus* (Peale), and *Ctenocephalides felis* (Curtis) *of domestic cats, Felis domestica* Linn.

Successful transmissions were obtained with *D. montanus, C. felis, N. fasciatus, X. cheopis* and *H. anomalus.* Only *one* of 3 mass trials with *H. anomalus* resulted in transmission indicating the possibility that this species may prove to be a rather poor vector.

To determine the vector efficiency experimentally, clean laboratory-reared fleas of each species tested were first fed upon an infected mouse in which the magnitude of bacteremia was known; thereafter each flea was given an opportunity to feed at daily intervals

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* This investigation was aided by a grant from the Rosenberg Foundation.

until death upon white mice. Infection of the fleas was determined by means of daily fecal cultures during the life of each flea and by subsequent histological examination. All experiments were conducted under the same laboratory conditions and employed the same strain of stock mice.

Individual feeding trials have demonstrated *D. montanus* to have an infection potential of .85, a vector potential of .52, and a transmission potential of 2.58 with a vector efficiency of 1.14. That is, of 48 fleas used 41 or 85% became infected; of these 41 infected fleas 21 or 52% became infective and transmitted the infection to 50 mice or an average of 2.58 transmissions per infective flea. Dividing the percentages by 100 the potentialities of a single flea may be obtained. In other words a group of *D. montanus* given an infectious blood meal will yield an average of 1.14 transmissions per flea. Similar individual feeding trials conducted with *X. cheopis* show this species of flea to have an infection potential of .98, a vector potential of .29, a transmission potential of 1.44 and a vector efficiency of .39 (Table I).

The vector efficiency relationship between the 2 species, as determined experimentally, should remain relatively constant; however, the actual figures obtained will undoubtedly vary depending upon the susceptibility of the species of laboratory animal used and climatic factors operating during the course of the experiment.

TABLE I.

Species	No. used	No. infected	No. infective	No. transmissions	Infection potential	Vector potential	Transmission potential	Vector efficiency
D. montanus	48	41	21	50	.85	.52	2.58	1.14
X. cheopis	50	49	14	20	.98	.29	1.44	.39

As shown in the table, although *X. cheopis* has a higher infection potential than *D. montanus,* only one-half as many *X. cheopis* become infective and these transmit but one-half as many times. Apparently then a high infection potential alone is no indication of high vector efficiency; likewise the vector potential alone without a consideration of the transmission potential will not give a true picture of vector efficiency. From the above data an accurate evaluation of vector efficiency must include a knowledge of the infection potential, the vector potential and the transmission potential.

13041

Effect of CO_2 on Ameboid Changes in Motor Nerve Plates in Intercostal Muscle.*

EBEN J. CAREY.

From the Department of Anatomy, Marquette University School of Medicine.

Protoplasmic movement has been proposed by Cajal[1] and many others as concerned in the functional activities at the synapses of the nervous system. He likewise discovered the growth cones on the terminals of embryonic and regenerating nerve fibers and attributed their extension to an ameboid movement of the growing axon. Harrison[2] demonstrated this protoplasmic movement of embryonic living nerve fibers in tissue culture which was confirmed by Lewis[3] and others.

That the variations in the size, shape, and internal structure of motor nerve plates in one structural and functional group of muscle fibers, in the same animal, during different degrees of intensity and rates of functional activity are likewise due to ameboid-like movements have not been experimentally demonstrated.

Method. The following simple experiment may be made to confirm the results reported in this paper which were repeated on 10 white rats. All of the photomicrographs (Figs. 1 to 17) were taken from the right intercostal muscles of rat No. 5. The rats were anesthetized by the intraperitoneal injection of nembutal, 25 mg per kilo. The hair was clipped with the electric clipper over the right dorsal aspect of the thorax. A one-inch vertical incision, 1 cm to the right of the mid-line of the spine, was made through the skin and muscle down to the ribs. The 6, 7, and 8 ribs and related intercostal muscles were quickly excised in one piece beginning medially 1 cm from the spine and extending 1.5 cm laterad. This excised part of the thoracic wall was run through the gold chloride technic and served as a control of the relatively normal motor end plates in the intercostal muscles of the right side. The bleeding was quickly controlled by hemostats and ligature. The skin was sutured

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* These investigations were carried out with the aid of a grant for research to the Department of Anatomy of the Marquette University School of Medicine by the Committee on Scientific Research of the American Medical Association and the National Foundation for Infantile Paralysis, Inc.

[1] Cajal, S. Ramon y, *Anat. Anz.*, 1890, **5**, 609.

[2] Harrison, R. G., PROC. SOC. EXP. BIOL. AND MED., 1907, **3**, 179; *Anat. Rec.*, **1**, 116; *J. Exp. Zool.*, 1910, **9**, 787.

[3] Lewis, W. H., *Am. J. Anat.*, 1907, **6**, 461.

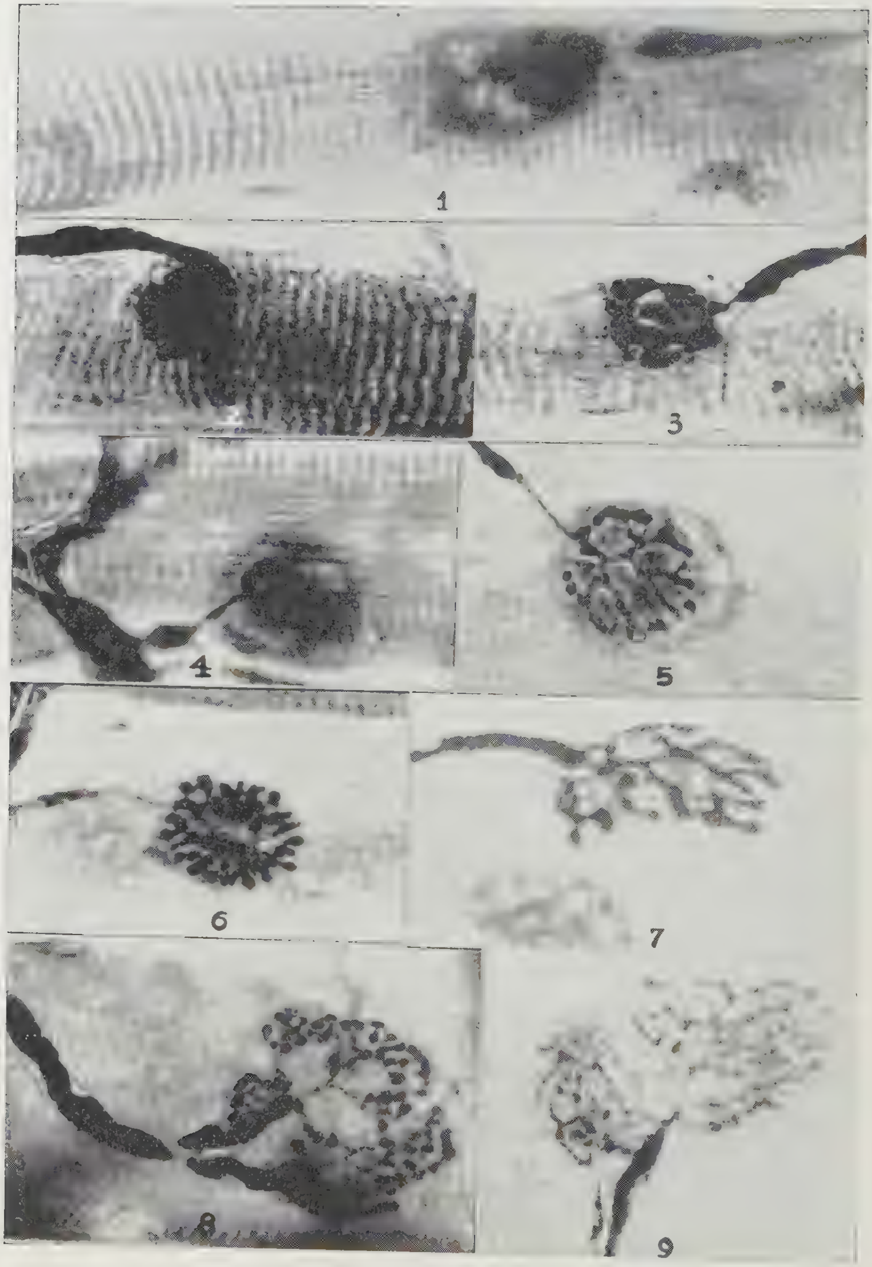

FIG. 1 TO 9.

Photomicrographs, teased normal motor nerve plates in intercostal muscle fibers, white rat No. 5, Fig. 1 to 7; carbon dioxide stimulation, Fig. 8 and 9, rat No. 5; gold chloride; × 700.

over the thoracic gap and the animal immediately placed for 2 to 10 minutes in a jar, the air of which contained roughly 15% of carbon dioxide. The rat was removed from the jar and the third and fourth intercostal muscles of the right thoracic wall were then quickly excised and run through the gold chloride technic for microscopic study of the teased muscle fibers and motor end plates. After again suturing the skin the animal was allowed to breathe normal air for a period of four hours and then decapitated. The ninth, tenth, and eleventh right intercostal muscles were then excised, subjected to the gold chloride technic, and teased.

Results. The respiratory rate after nembutal anesthesia in the white rat varied from 30 to 50 per second. During excision of the piece from the right thoracic wall the rate increased to that of 60 to 80 per minute. This was the reason for making the first incision through the ribs along the dorsal aspect, thereby quickly severing the nerve supply to the excised muscles before the respiratory rate increased. These muscles were used as relatively normal controls. The rat was then placed in carbon dioxide. The respiratory rate varied from 130 to 150 per minute. When the rate of labored breathing fell below 100 per minute the rat was taken out of the jar. The second excision of the right third, fourth, and fifth ribs was quickly made. The respiratory rate fell to 60 per minute within 30 minutes while the rat breathed normal air and remained at this rate until the animal was decapitated 3½ hours later. By this simple means the histologic changes in the same group of muscle motor nerve plates in the same animal were observed under different degrees of intensity of functional activity stimulated by the chemical method of carbon dioxide.

The motor nerve plates from the right intercostal muscles in the relatively normal controls excised prior to carbon dioxide stimulation varied from the retracted state with wide fronds (Fig. 1), 25 microns in diameter to the expanded state (Fig. 7), 52 microns in diameter. These were the extremes in the dimensions of 1000 normal nerve plates. The retracted motor end plates with wide coarse fronds are related to coarse widely spaced cross striations (Figs. 1 to 3). The expanded plates with narrow elongated processes are related to fine, closely-spaced cross striations (Figs. 5 to 7). This finding confirms the classification of muscle fibers by Hines[4] into coarsely and finely striated. There was a closely graded series of transitional stages in the structure of the muscle fibers between these two extremes with variations in the number of

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[4] Hines, M., *Am. J. Anat.*, 1931, **47**, 1.

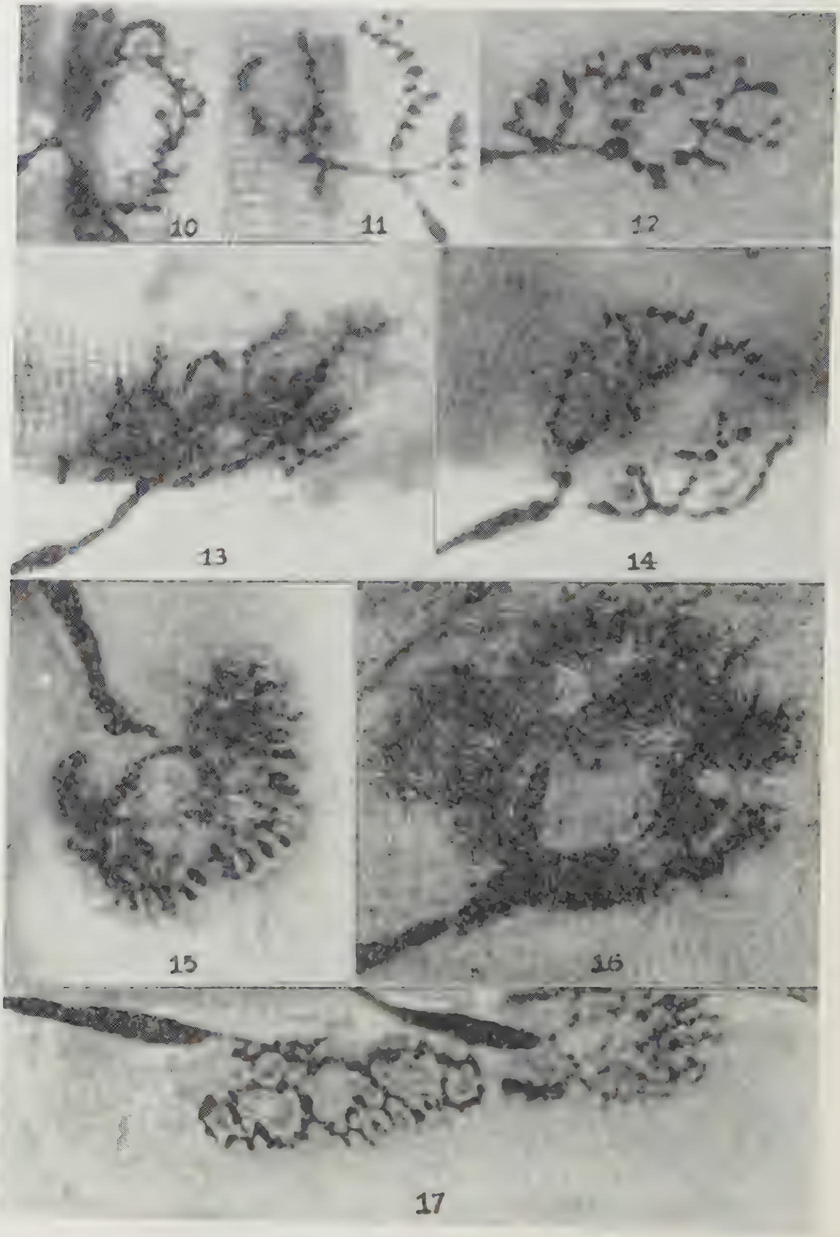

FIG. 10 TO 17.
Photomicrographs, teased motor nerve plates in intercostal muscle fibers, white rat No. 5, stimulated with carbon dioxide; gold chloride; × 700. Ameboid expansions of processes of end plate in respiratory superactivity.

muscle cross striations which agreed with the observations reviewed by Cobb,[5] Needham,[6] Hines,[7] and Hinsey.[8]

The motor nerve plates from the right intercostal muscle stimulated by carbon dioxide to superactivity had in 1000 measurements 341 plates that varied from 30 to 58 microns in diameter. The largest motor nerve plates expanded by carbon dioxide stimulation of the respiratory center reached a diameter of 105 microns (Fig. 16). In 1000 measurements of the diameters of stimulated motor nerve plates, 659 varied from 58 to 87.5 microns and had 2 to 6 elongated, discrete processes with periodic enlargements. These long, attenuated processes were separated by wide meshes (Figs. 8 to 17) and related to fine, closely spaced cross striations. In some places the protoplasmic projections of the end plates formed anastomoses (Figs. 12 and 17). There were 2 motor nerve plates on one muscle fiber; these were the 2 terminals of the 2 medullated branches of a single axon (Fig. 17). The sole plate of Kühne formed a dark granular rim around the retracted and slightly expanded normal motor plates (Figs. 1, 4, and 5). The granular sole plate disappears in the greatly expanded end plates related to fine, closely-spaced striae (Figs. 9 and 17). There was a reduction by 80% in the number of motor nerve plates expanded beyond 55 microns in diameter in the right 9, 10, 11 intercostal muscles after rat No. 5 was again breathing normal air for 3½ hours. During this time the respiratory rate fell from 150 to 60 per minute.

Summary. The retracted motor nerve plates with wide, short projections were related to coarse, widely spaced cross striations, whereas expanded nerve plates with thin, elongated and moniliform processes were longer and separated by wider meshes than the retracted ones. 60% of the expanded motor nerve plates in the intercostal muscle accelerated in the transmission rate of the nervous impulses by carbon dioxide became twice the size of the relatively normal plates. Their processes were longer and separated by wider meshes than those of the normal controls. This morphologic expansion and extension of the processes of the plate by the proposed ameboid motion under the stimulus of the demand of increased functional activity evidently favors the transmission of the nerve impulse to the receptive muscle substance by increase of the surface area of the motor nerve plate. The differences in the histologic structure of motor nerve plates and muscle cross striations may be arranged in a graded series and are evidently determined by the active and inactive fibers in the fractional contraction of the same muscle. These structural changes favor the chemical theory of impulse transmission.

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[5] Cobb, S., *Physiol. Rev.*, 1925, **5**, 518.

[6] Needham, D. M., *Physiol. Rev.*, 1926, **6**, 1.

[7] Hines, M., *Quart. Rev. Biol.*, 1927, **2**, 149.

[8] Hinsey, J. C., *Physiol. Rev.*, 1934, **14**, 514.

13042

Effect of Angiotonin and Renin on Glomerular Circulation in Frog Kidney.

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Angiotonin is the substance produced by the interaction of renin and renin activator in the plasma.[1, 2, 3] Corcoran, Kohlstaedt and Page expressed the opinion that angiotonin is a highly active vasoconstrictor which raises the blood pressure without simultaneously depressing the cutaneous temperature. They reported the effect of angiotonin on the kidney to be predominantly a vasoconstriction of the efferent arterioles of the glomeruli.[4, 5, 6] Hill and Andrus[7] noted an increase in the amplitude of the heart beat, a decrease in coronary flow, but no consistent effect on the rate of the perfused heart of the cat when angiotonin was added to the perfusate. However, renin had no significant effect. Page and Helmer[8] observed tolerance to successive doses of angiotonin in normal dogs, but if the kidneys are removed, the degree of tolerance becomes slight.

The purpose of this investigation was to study by direct microscopic observation the effect of angiotonin* and of renin* on the

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[1] Kohlstaedt, K. G., Page, I. H., and Helmer, O. M., *Am. Heart J.*, 1940, **19**, 92.

[2] Kohlstaedt, K. G., Helmer, O. M., and Page, I. H., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 214.

[3] Page, I. H., and Helmer, O. M., *J. Exp. Med.*, 1940, **71**, 29.

[4] Corcoran, A. C., Kohlstaedt, K. G., and Page, I. H., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 244.

[5] Corcoran, A. C., and Page, I. H., *J. A. M. A.*, 1941, **116**, 690.

[6] Corcoran, A. C., and Page, I. H., *Am. J. Physiol.*, 1940, **130**, 335.

[7] Hill, W. H. P., and Andrus, E. C., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 213.

[8] Page, I. H., and Helmer, O. M., *J. Exp. Med.*, 1940, **71**, 495.

* The authors wish to express their gratitude to the directors of the Eli Lilly Laboratories who, through Dr. Corcoran, have generously supplied all the angiotonin and renin needed for this study.

renal circulation by transilluminating the kidney in the intact frog.

Method. For the study of the effect of angiotonin and of renin on the circulation in the kidney of the frog, a modification of the method of illumination described by Knisely[9, 10] was used. The experiments were made on anesthetized male frogs *(Rana pipiens)* whose weights ranged from 25 to 45 g. Anesthesia was produced by injecting into the dorsal lymph sac 0.1 cc of a 25% solution of urethane per 10 g of body weight. The abdomen of the frog was opened by a paramedian incision, carefully avoiding the large vessels, and the edges of the incision were cauterized gently to prevent loss of blood. The animal was laid on a block of cork with frog Ringer's solution gently flowing over the exposed viscera to prevent drying. Water was kept trickling around the intact body of the frog to dilute the Ringer's solution in contact with the skin and prevent dehydration. The kidney was protected from the heating effect of the light, transmitted through the quartz tubing, by a constant stream of Ringer's solution flowing through the bore in the delivery tip introduced retroperitoneally, dorsad to the kidney. Such a preparation could be kept functioning normally for at least 9 hours. Healthy frogs were used and the normal renal circulation in several microscopic fields was studied carefully before any observations on the effects of angiotonin or of renin were made.

Amounts of angiotonin or of renin ranging from 0.01 to 0.1 cc were injected either into the lymph sacs or intravenously into the abdominal vein, or were applied directly through a very fine needle to the microscopic field under observation. A stop watch was used to record the time when the drug was administered, when any effect was observed, and when the affected parts returned to their original condition previous to the administration of the drug.

Results. Angiotonin. By direct application of one or 2 drops of undiluted angiotonin over the region under microscopic observation a transient cessation of circulatory activity of the glomeruli, with moderate blanching of the region, was produced within 10 to 15 seconds and lasted for a fraction of a minute. This was followed soon by moderate packing of blood corpuscles and slight engorgement in the glomeruli which lasted for more than 8 minutes. Thereafter the renal circulation gradually returned to the preadministration state. This was consistently true for the first 4 or 5 administrations, but any application after that produced neither blanching nor cessation of activity in the glomeruli; nevertheless, the slight

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[9] Knisely, M. H., *Anat. Rec.*, 1936, **64**, 499.

[10] Knisely, M. H., *Anat. Rec.*, 1938, **71**, 503.

engorgement of the glomeruli and the increase in the activity of the heart were produced repeatedly.

Injection of 0.025 to 0.1 cc of undiluted angiotonin into the submaxillary lymph sac caused, within 2 minutes, very slight paling of the region of the kidney under observation. This was followed soon by moderate engorgement of the glomeruli for more than 10 minutes. Within 15 minutes the glomerular, as well as the rest of the renal, circulation under observation gradually returned to the preinjection state. The injection of angiotonin into the lymph sac did not cause cessation of glomerular circulation.

Intravenous administration of 0.025 to 0.1 cc of undiluted angiotonin within 15 seconds caused blanching of the region and cessation of circulatory activity in the glomeruli which lasted for about one minute. However, this was followed soon by increase in circulatory activity in the kidney and moderate engorgement of the glomeruli. This engorgement phase lasted for about 8 minutes, after which the renal circulation gradually returned to the preinjection state.

When angiotonin was administered repeatedly after the effect of a previous injection had worn off, it was observed that only moderate engorgement resulted, but no blanching was produced even with increased dosage, no matter what route was used for the administration. Under the influence of angiotonin it was observed that the heart rate was increased about 10 beats per minute and the force of the heart beat was pronounced. This is in agreement with the observation of increased amplitude of the beat of the perfused heart of the cat as reported by Hill and Andrus.[7]

In comparing the effect of angiotonin with that of epinephrine, it was observed that the vasoconstriction and blanching as well as the subsequent engorgement of the glomeruli produced by a 1:100,000 dilution of epinephrine were much more marked than those produced by undiluted angiotonin but the duration of the engorgement caused by epinephrine was shorter.

Renin. Whether directly applied or injected into the lymph sac or intravenously, renin produced no demonstrable change in the renal circulation of the frog *(Rana pipiens)*.

Summary. Angiotonin, when applied directly or administered intravenously produced transient vasoconstriction with cessation of activity of the glomerular circulation for about one minute. This was followed by moderate engorgement of the glomeruli which lasted about 8 minutes. When angiotonin was administered subcutaneously, moderate engorgement of the glomeruli was produced without an initial cessation of glomerular circulatory activity. With successive administrations of angiotonin, the blanching effect disappeared but the glomerular engorgement was observed consistently.

Renin had no detectable effect on the renal circulation in the frog.

13043 P

Agglutination of Encephalitis Virus-Coated-Bacterial Cells by Virus Antisera.

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Details of the technic involved in satisfactorily coating bacterial cells with serum antigen and the use of such cells in an agglutination test have been given in a previous report.[1] A particular advantage of the procedure is that very minute amounts of serum antibody specific for the adsorbed antigen can be detected, even though the amount of antibody is too small to be revealed by the conventional precipitation test. The use of this very sensitive type of an antigen-antibody reaction, for the detection of 'antibodies' in the blood of man and of animals against the virus of St. Louis type of encephalitis, is the basis of the present report.

The microörganisms *(Serratia marcesens)* were prepared for use in the agglutination reaction by incubating them with virus as contained in a saline emulsion of infected mouse brain. That virus was adsorbed by the bacteria in significant quantity was indicated by the observation that such cells, after repeated washing with saline, would subsequently reproduce the disease in mice upon intranasal implantation.* Suspensions of virus-coated bacteria were employed as antigens in making agglutination tests with the sera of (a) human beings convalescent from encephalitis, (b) normal individuals, and (c) of rabbits immunized against encephalitis virus.

With the sera of convalescent patients† agglutination reactions occurred in dilutions ranging from 1:16 to 1:128. The specific nature of the agglutination reaction was indicated by the observation that after adsorption of these sera with virus-coated bacterial cells they failed to elicit the agglutination reaction when tested against additional preparations of virus-coated cells. On the other hand, sera adsorbed with bacterial cells coated with normal mouse brain were capable of agglutinating virus-coated cells to the original titer. In addition, similar adsorption of these sera with the brain substance of normal mice did not remove the capacity of the sera to react with cells coated with mouse brain-virus. Although agglutination was observed in all cases, the titers of sera of convalescent patients were not in complete agreement with their virus neutralizing capacities (as determined by mouse protection tests). Further observations are required to clearly indicate this relationship.

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[1] Roberts, E. C., and Jones, L. R., Proc. Soc. Exp. Biol. and Med., 1942, **47**, 11.

* Subsequent testing of the brains of such infected mice established the specific identity of the virus.

† For supplies of sera and for data pertaining to mouse neutralization tests we are indebted to Dr. G. O. Broun of the Department of Medicine of St. Louis University.

The bacterial agglutination (B.A.) method of detecting encephalitis virus antibody has been applied likewise to the blood sera of a group of 18 supposedly normal individuals, wherein it was found that the sera of 80% of those tested gave a reaction in dilutions of 1:8 or higher. The results of the B.A. method of antibody detection were correlated in most instances with the presence of neutralizing antibodies, although the neutralizing capacities were not in complete agreement with the B.A. titers. A more extensive study of the incidence of antibodies in the sera of normal individuals, as revealed by the B.A. method is under way.

The B.A. method of antibody detection was similarly applied to two lots of rabbit antisera which had been prepared by immunization of the animals with mouse brain-encephalitis virus suspensions.[†] These antisera gave B.A. reactions in dilutions of 1:512. The specific nature of the reaction was indicated by the failure of normal rabbit sera to elicit the B.A. reaction and by differential adsorption of the rabbit immune sera with (a) normal mouse brain (which did not remove the antibody involved in the B.A. reaction) and (b) encephalitis virus-mouse brain (which completely removed the antibody involved). These antisera, when adsorbed with bacterial cells coated with normal mouse brain, were capable of agglutinating virus-coated cells to full titer. On the other hand, immune rabbit sera adsorbed with bacterial cells coated with mouse brain-virus were devoid of agglutinating antibody.

Additional experiments are under way seeking to make a further application of the B.A. method to the detection of humoral antibody involved in experimental poliomyelitis of animals and in the naturally occurring disease of man.

13044 P

Use of Synthetic Medium in Study of Antibacterial Effect of Sulfathiazole.*

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In the present study we have employed the synthetic medium previously reported as being valuable for the study of the antibacterial effect of the thiazole compounds against *Salmonella enteritidis* and, as originally employed in demonstrating the inhibiting effect of p-aminobenzoic acid upon these drugs.[1] This liquid medium contains the same nutritive materials as are present in Simmon's citrate agar and is quite suitable for the cultivation of this organism even though very small inocula are employed.

This synthetic medium possesses definite advantages over ordinary broth containing meat extractives and peptone, since it is invariably uniform in composition, devoid of growth factors, essential metabolites, and of such substances as are known to interfere with the activity of the sulfonamide drugs. That this synthetic medium is superior to ordinary broth is further revealed by our experimental observations wherein 0.3 mg % of sulfathiazole prevents growth

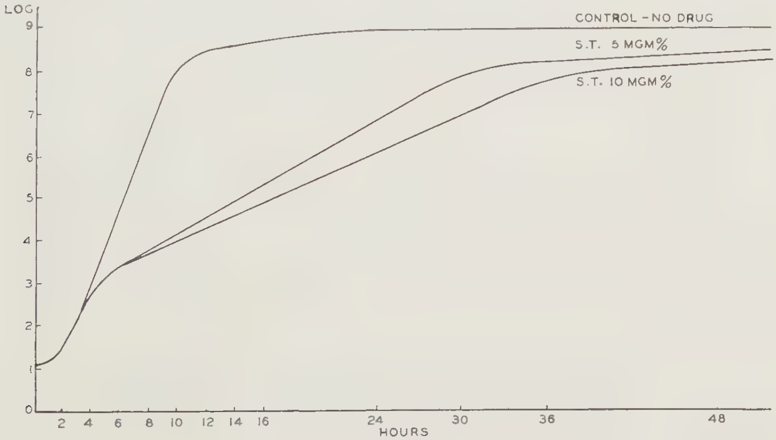

Fig. 1.

Viable cell counts of *S. enteritidis* in meat extract-peptone-broth with and without sulfathiazole.

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* Aided by a grant from the Department of Medical Research of the Winthrop Chemical Company.

[1] Muir, R. D., Shamleffer, V. J., and Jones, L. R., *J. Bact.*, 1941, **41**, 84.

(as judged by visible turbidity) of *S. enteritidis* in the former whereas 20 mg % does not prevent growth in the latter medium.

Recognizing this superiority, it was deemed of interest to ascertain the relative effects of sulfathiazole upon *S. enteritidis* in each of these media as would be elicited by a comparison of viable population curves. In Fig. 1 are recorded the growth curves of the organism in meat extract-peptone broth, without drug, and with 5 and 10 mg % sulfathiazole. One of the effects of sulfathiazole is seen as a delay in the inception of the logarithmic phase of multiplication. During this phase, the drug (5 mg %) serves to increase the generation time by about five-fold as is reflected in the slope of the curve. The stationary phase is reached much earlier in control cultures. However, the ultimate number of cells developing in the drug-containing media is about one-fourth the number found in the control.

In Fig. 2 are recorded curves, similarly developed from cultures in synthetic medium. In a comparison of the control cultures and sulfathiazole cultures, it will be noted that the rate of multiplication does not appear to be markedly inhibited during the first 6 or 8 hours, whereas subsequent to that time while the control culture continues to increase logarithmically for the next 24 hours, the sulfathiazole cultures tend to remain stationary for an extended period. In the sulfathiazole cultures, during the interval from the 4th to the 8th hour there is an ill-defined period of probable logarithmic increase. In the stationary phases the control culture populations were about 150,000 times those observed in the sulfathiazole cultures.

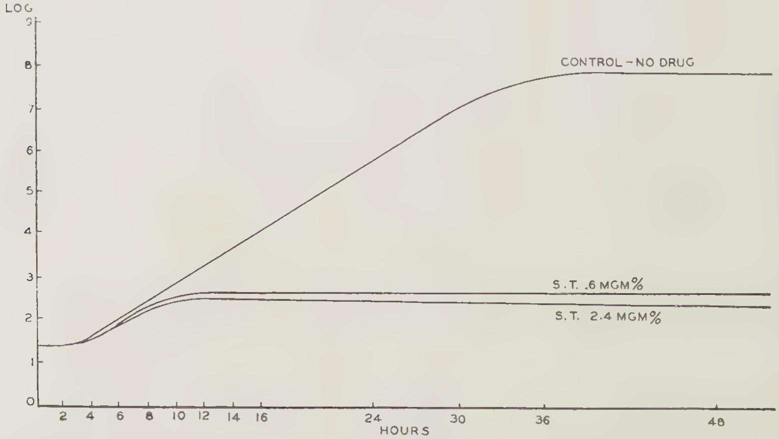

FIG. 2.

Viable cell counts of *S. enteritidis* in synthetic medium cultures with and without sulfathiazole.

In a comparison of Fig. 1 with Fig. 2, it will be noted that with peptone broth 5 and 10 mg % were employed whereas 0.6 and 2.4 mg % were used in the synthetic medium. In peptone broth sulfathiazole serves to reduce bacterial proliferation by about 75% whereas a much smaller quantity of sulfathiazole in synthetic medium serves to reduce bacterial multiplication by more than 99.9%. From these figures it becomes evident that sulfathiazole is approximately ten times as effective in the synthetic medium as it is in meat-extract peptone broth.

13045

Histopathology of CNS of Mice Infected with Virus of Theiler's Disease (Spontaneous Encephalomyelitis.)*

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Since 1937, when Theiler[1] first described spontaneous encephalomyelitis of albino mice (Theiler's disease; "mouse poliomyelitis") and the virus causing it, two major developments have given this disease renewed importance because they confirm the impression that one is dealing here with an infection more closely related to human poliomyelitis than is any other known disease. In the first place, the finding by Olitsky[2] and by Theiler and Gard[3] of Theiler's virus in the intestinal contents and feces not only of mice showing the signs of the spontaneous or experimental malady but also of normal, young adult or mature animals, recalls similar results obtained in studies on human poliomyelitis.[4, 5] Again another epidemiological feature is shown commonly by the two diseases, *i. e.*, the analogous incidence of paralytic cases of one in more than 5000.

The second recent development under consideration concerns the successful transmission by Armstrong[6] of one strain (Lansing)

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* The writers wish to thank Dr. M. Theiler for his coöperation and for Lansing-strain infected mice, and Mr. P. Haselbauer for his technical assistance.

[1] Theiler, M., *J. Exp. Med.*, 1937, **65**, 705.

[2] Olitsky, P. K., Proc. Soc. Exp. Biol. and Med., 1939, **41**, 434; *J. Exp. Med.*, 1940, **72**, 113.

[3] Theiler, M., and Gard, S., *J. Exp. Med.*, 1940, **72**, 49, 79.

[4] For literature see Trask, J. D., and Paul, J. R., *Am. J. Public Health*, 1941, **31**, 239.

[5] Sabin, A. B., and Ward, R., *J. Bact.*, 1941, **41**, 49.

[6] Armstrong, C., *Public Health Rep.*, 1939, **54**, 1719.

of human poliomyelitic virus to the white mouse. This notable achievement enables one now to carry the comparison of the 2 diseases further than it was heretofore possible. The clinical resemblance of human and experimental (monkey) poliomyelitis on one side and mouse-encephalomyelitis on the other has been fully stressed before.[1–3] In the mouse, the two maladies are clinically indistinguishable.

In view of the striking similarities noted in many properties of the two diseases, it was thought desirable to study in detail the histopathology of spontaneous and experimental Theiler's disease (a) in the hope that clues might be yielded which in turn could be applied to problems in the pathogenesis of human poliomyelitis and (b) for the purpose, particularly, of comparing in the same host the pathology of the Lansing strain of poliomyelitis and of Theiler's disease.

Methods and Materials. The CNS of more than 30 mice sacrificed either at the height of spontaneous Theiler's disease, or at various stages of the experimental infection following intracerebral *(regio parietalis),* intranasal, intralingual, and intraäbdominal inoculation of the virus deriving from intestinal contents of mice were studied. Virus introduced by peripheral routes induced paralysis in approximately 10 to 15% of inoculated 14-day-old mice. In most instances the entire head from which the skin and lower jaw had been removed, and the entire spinal column were fixed in Zenker's solution plus 10% glacial acetic acid (for decalcification) for 30 hours before washing. The skull and contents were cut semiserially, each sagittal section being 6 microns thick; the first 6 sections of each ribbon of 30 were retained for mounting. The spinal column was cut only at the middle of the cervical, thoracic, and lumbar levels. Thus about 150 sections of the CNS were cut from each animal, and more than 5000 in all were studied. Eosin-methylene-blue or hematoxylin-eosin stains were used.

This method was first employed by Sabin and Olitsky[7] who showed thereby the definite and selective pathways taken in the mouse, from the site of inoculation through the CNS, by another neurotropic virus, vesicular stomatitis. The progression of poliomyelitic virus through the CNS of man and monkey has been delineated by Sabin,[8] Bodian and Howe,[9] and others.[8b, 9]

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[7] Sabin, A. B., and Olitsky, P. K., *J. Exp. Med.,* 1938, **67**, 201.

[8] a. Sabin, A. B., and Olitsky, P. K., *J. Am. Med. Assn.,* 1937, **108**, 21; b. Sabin, A. B., *Am. J. Dis. Child.,* 1940, **60**, 1313.

[9] Bodian, D., and Howe, H. A., *Brain,* 1940, **63**, 135.

Results of Microscopical Examination. We have not been able, however, to find such clear-cut patterns in the brain of mice having Theiler's disease. Instead, no significant differences in the types of lesion and their distribution were noted, regardless of the route of inoculation. Of particular interest is the fact that the olfactory bulbs exhibited no characteristic changes—either in those 8 mice receiving the virus intranasally and responding with paralysis, or in 22 others which were infected by other routes, or in the spontaneous disease. This is in agreement with what is found to be true in human poliomyelitis but is in sharp contrast to the bulb-lesions that characterize poliomyelitis in the monkey following intranasal instillation of virus.[8a] In this relation it should be pointed out that human poliomyelitis and murine encephalomyelitis are natural diseases of man and mouse but monkey-poliomyelitis is an experimental infection, artificially induced. Other pathways that may, possibly, be taken by intranasally instilled Theiler's virus are via the V nerve or the sphenopalatine (parasympathetic) or superior cervical (sympathetic) ganglia. It was noted, however, that the gasserian and superior cervical ganglia were affected not only after intranasal but also after intralingual and intracerebral inoculation of virus. In no instance have we been able to find lesions in the sphenopalatine ganglion.

Intracerebral injection of intestinal Theiler's virus in sufficient dosage was invariably followed by clinically apparent infection with an incubationary period extending from 7 to 37 (usually 10 to 20) days.[2] Animals so inoculated were sacrificed after 7 days, before clinical signs of infection (paralysis) were seen. In the brains, the most marked lesions were observed at the site of injection. The area immediately surrounding it showed endothelial swelling and proliferation chiefly of the smaller vessels and capillaries, and perivascular microglial and lymphocytic infiltration. Perivascular neuronal degeneration and, less often, necrosis also were present. The lesions extended from the site, rostrally to the septal area and caudally to the pontine level. They spread out mainly periventricularly.

In still later stages of the period of incubation after intracerebral inoculation (9th-11th day), the lesions had expanded over the adjacent structures but chiefly periventricularly. The vascular changes as mentioned were more in evidence, as was perivascular gliosis, the gliosis also occurring elsewhere, diffusely or focally. Neuronal degeneration and necrosis were more marked but neuronophagia was met with only infrequently and the cells active in this process were neuroglial rather than polymorphonuclear. The pathological changes rostrally, at the septum or *corpus striatum* were not extensive and were predominantly of mesodermal-glial type; caudally, from the level of the thalamus to that of the *substantia nigra* or pons-medulla, they were more prominently neuronal (degeneration, necrosis; neuronophagia; loss of neurons with resulting vacuolization of the stroma), while the mesodermal-glial reactions became less conspicuous (Fig. 1).

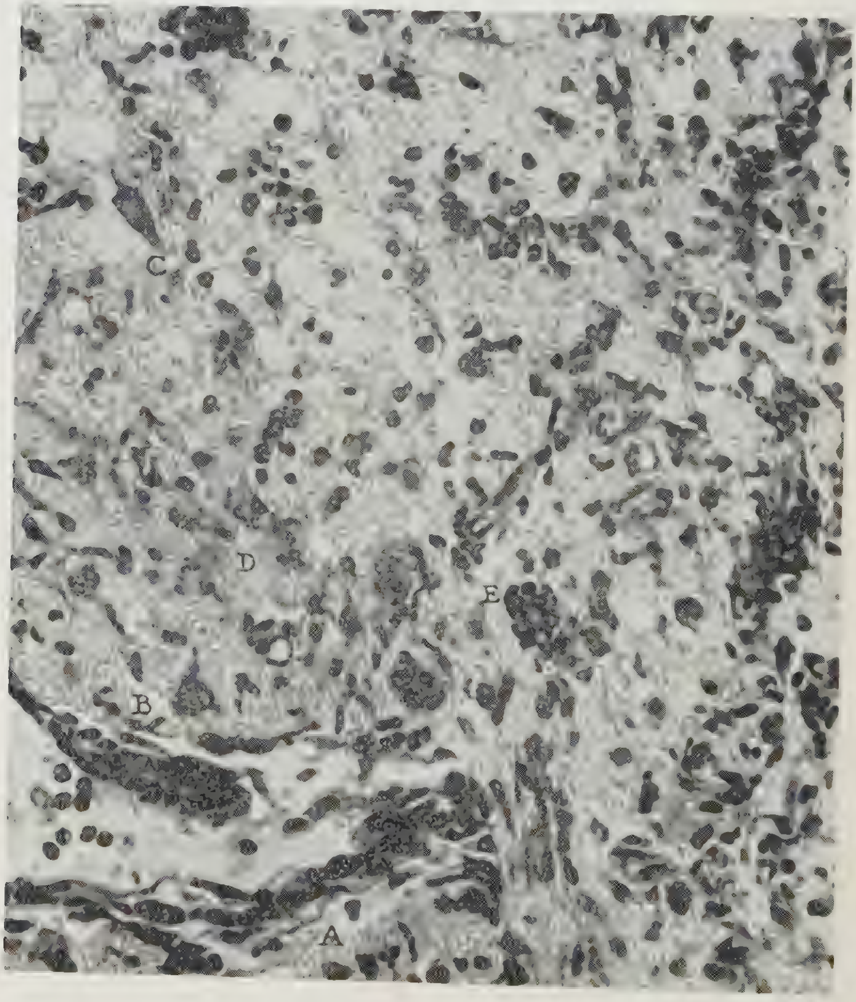

FIG. 1.

Substantia nigra in encephalomyelitis of the mouse. To be noted are the endothelial (A) and perivascular (B) reaction and neuronal degeneration (C), necrosis (D), neuronophagia (E) and vacuolization of the stroma. × 500.

Thus, at this stage, when clinical signs were still absent and the cord was still free from detectable changes, the characteristic picture of the fully developed disease was noted in the brain, except that in the latter stage the degree of reactions were more pronounced. The areas then most constantly affected were the *substantia nigra, tegmentum,* reticular formation, olivary nuclei, nuclei of V and VIII nerves, red and dentate nuclei. Cortical lesions were few in number, mainly vascular in type and sometimes consisted of neuroglial infiltration.

After onset of paralysis, in addition to the cerebral lesions, the anterior horns of the spinal cord were regularly involved: They revealed the vascular and perivascular reactions, neuronal degeneration, necrosis, and neuronophagia. The neuronal changes, however, were never as extensive as they are ordinarily in the monkey cords in experimental poliomyelitis—there was always a number of apparently normal neurons present and only a few polymorphonuclear cells were, as a rule, seen. It is of interest that certain neurons showed, usually at the onset of paralysis and chiefly in the anterior horns of the cord, the presence of Cowdry Type B, intranuclear inclusion-bodies, similar to those seen in human and monkey poliomyelitis.

The progression of pathological changes during the period of incubation following peripheral inoculation of Theiler's virus could not be studied in the same way, in view of the irregularity of production of clinically apparent infection by these means.

After peripheral inoculation when paralysis developed, however, the pathological picture differed in no significant manner from that seen after intracerebral injection (except for the more pronounced periventricular distribution in the latter), and was also similar to that shown by mice spontaneously attacked.

In conclusion, in the fully developed clinical disease the types of lesions and their distribution in the CNS were not essentially different when the malady was induced by various routes of inoculation, regardless of the length of incubationary period. As a rule, the rostral part of the brain showed mesodermal-glial reactions predominantly, while the caudal, including the cord, exhibited more marked neuronal degeneration and destruction.

The type of lesions and their distribution in Theiler's disease in albino mice closely resembled the picture in this animal at corresponding periods after infection with the Lansing strain of human poliomyelitis.[10]

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[10] Lillie, R. D., and Armstrong, C., *Public Health Rep.*, 1940, **55**, 718.

13046

Metabolism of Pathogenic Clostridia in Complex Carbohydrate-Rich Culture Media.*

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One of the characteristics of the pathogenic and many of the non-pathogenic clostridia is their marked ability to digest and further metabolize proteins and protein derivatives during growth in the usual culture media.[1–5] Ammonia, volatile acids, CO_2, and H_2 are produced during the anaerobic breakdown of proteins and amino acids.[1–7] Carbohydrates are the source of most of the energy in carbohydrate-rich media,[†] and large quantities may be consumed, due to the formation of neutral compounds, CO_2 and H_2. Dextrose yields ethyl alcohol and formic (CO_2 and H_2), acetic, butyric, and lactic acids.[2, 8–11] In the later stages of the fermentation the commercially important clostridia may yield very large quantities of acetone, and iso-propyl and butyl alcohols.[3, 9, 12] The same products may be obtained from lactic and pyruvic acids.[13, 14, 15] Acetic acid may be converted into acetone and butyl alcohol.[12] The presence of acetone and the higher alcohols in cultures of pathogenic clostridia has not been reported. Acrolein is found in cultures of *Cl. perfringens (welchii)* in glycerol broth.[16] The presence of acrolein in fruit juices and the bitterness of wine have been attributed to the growth of this organism.[17] Acetone may be reduced to iso-propyl alcohol;[18] aldehydes and acids are reduced to alcohols;[2, 15, 18, 19, 20] and acetaldehyde may yield acetylmethyl carbinol.[20] The clostridia thus appear to be versatile in their abilities to carry out chemical reactions with a wide variety of substances.

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* This study was supported by the Bartlett Memorial Fund and by the Douglas Smith Foundation for Medical Research of the University of Chicago.

[1] Hall, I. C., and Finnerud, F., Proc. Soc. Exp. Biol. and Med., 1921, **19**, 48.

[2] Speakman, H. B., *J. Biol. Chem.*, 1926, **70**, 135.

[3] Peterson, W. H., and Fred, E. B., *Ind. Eng. Chem.*, 1932, **24**, 237.

[4] Wolf, C. G. L., and Harris, J. E. G., *J. Path. Bact.*, 1918-1919, **22**, 1.

[5] Wolf, C. G. L., *J. Path. Bact.*, 1918-1919, **22**, 115.

[6] Parsons, L. B., and Sturgis, W. S., *J. Bact.*, 1927, **14**, 181.

[7] Stickland, L. H., *Biochem. J.*, 1935, **29**, 889.

[†] Wolf and Harris[4] believe that the volatile acids produced by *Cl. histolyticum* in cooked meat (without added sugar) medium are derived from glucosamine of the meat.

[8] Speakman, H. B., and Phillips, J. F., *J. Bact.*, 1924, **9**, 183.

[9] Freiburg, G. W., Proc. Soc. Exp. Biol. and Med., 1925, **23**, 72.

[10] Sjolander, No. 0., *J. Bact.*, 1937, **34**, 419.

[11] Sievers, H., and Muller, E., *Z. Biol.*, 1927, **86**, 527.

[12] Speakman, H. B., *J. Biol. Chem.*, 1920, **41**, 319.

[13] Peterson, W. H., and Johnson, M. J., *J. Bact.*, 1933, **25**, 69.

[14] Brown, R. W., Osburn, O. L., and Werkman, C. H., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 203.

[15] Bernhauer, K., Iglauer, A., Groag, W., and Kottig, R., *Biochem. Z.*, 1936, **287**, 61.

A comparative quantitative study of the metabolism of the pathogenic clostridia under identical conditions of growth has never been made, nor have the principal products of metabolism in the usual liquid media with added dextrose been determined. In the present paper, data from 5 species of pathogenic clostridia are presented.

Experimental. The bacteriological and chemical methods were the same as described previously.[21] The inoculated media, con-

TABLE I.

The culture medium was prepared from fresh infusion of beef muscle (A), or meat extract (B), to which were added 1.0% of Difco Proteose-peptone (P), or Witte-peptone (W), 1.8% of $Na_2HPO_4 \cdot 12\ H_2O$, and 0.9% of dextrose. The initial reaction was pH 7.6.

Organism	Culture medium	Incubation 37° Hr	Dextrose consumed × 2 mM C_3 per l.	Lactic acid mM per l.	Formic acid mM per l.	Acetic acid mM per l.	Ethyl alcohol mM per l.	Butyric acid mM per l.	Undetermined acidity cc N per l.	Yield lactic acid %
Cl. perfringens	A_5W	10	29.0	9.5	12.1	7.3	4.7	0.7	2.2	33
(welchii)	B_6P	24	57.4	30.2	19.0	9.0	9.4	1.2	—1.2	53
Cl. septicum	A_5W	10	22.5	1.3	13.5	6.3	3.9	2.7	0.7	6
(Vibrion septique)	B_6P	24	49.7	13.6	25.7	12.3	13.1	3.1	1.0	27
Cl. sporogenes	A_5W	10	17.4	0.3	9.5	7.2	5.0	0.8	—0.6	2
	B_6P	24	47.6	4.1	27.1	12.5	23.0	2.0	—2.4	9
'' *tetani*	A_5W	10	11.9	—0.6	5.5	5.7	4.2	1.5	—3.9	loss
'' *botulinum*, Type A	A_5W	10	14.4	—4.1	6.1	5.5	5.2	1.3	—1.4	loss
'' '' '' ''	B_6P	24	21.9	2.5	15.6	11.7	8.9	2.2	—6.9	11
'' '' '' B	A_5W	10	19.5	0	14.9	10.2	6.5	1.0	—1.8	0
'' '' '' ''	B_6P	24	29.5	0.6	25.6	17.0	12.2	1.5	—6.4	2

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[16] Humphreys, F. B., *J. Infect. Dis.*, 1924, **35**, 282.

[17] Warcollier, G., and Le Moal, A., *Compt. Rend.*, 1932, **194**, 1394.

[18] Langlykke, A. F., and Fred, E. B., *J. Bact.*, 1937, **33**, 102.

[19] Blanchard, K. C., and MacDonald, J., *J. Biol. Chem.*, 1935, **110**, 145.

[20] Osburn, O. L., *Iowa State College J. Sci.*, 1935-1936, **10**, 97.

[21] Friedemann, T. E., *J. Bact.*, 1938, **35**, 527.

tained in tall, 4 oz dispensing bottles, were incubated from 10 to 24 hours at 37° to 38° in an atmosphere of pure nitrogen. Growth was stopped by the addition of exactly 1/10 volume of N H_2SO_4. Subcultures and smears were made just before acidification to determine the purity of the cultures. The organisms were obtained through the courtesy of Dr. G. M. Dack of the Department of Bacteriology.

The changes effected by growth of the organisms in two batches of culture media, differing somewhat in composition, are shown in Table I. The composition of the media is given at the top of the table.

Discussion of Results. The principal feature of the results shown in Table I is the large yield of formic acid, which, in most instances, tended to approach the sum of the mM of acetic acid and ethyl alcohol. *Cl. perfringens (welchii),* in culture medium A5W, consumed 14.5 mM of dextrose, or 29.0 mM of C_3, and yielded 12.1 mM of formic acid, and 12.0 mM of C_2-compounds. In the same batch of culture medium and during the same period of incubation *Escherichia coli* consumed an average of 26.9 mM of dextrose (or 53.8 mM of C_3) and yielded an average of 25.7 mM of formic acid, 15.4 mM of acetic acid, and 14.8 mM of ethyl alcohol. *Cl. perfringens* yielded 19.0 mM of formic acid and 18.4 mM of C_2-compounds in batch B6P. The results from *Cl. septicum (Vibrion septique)* were almost as regular. Slightly less formic acid than C_2-compounds was obtained from the other clostridia. No evolution of "gas" was seen when *Salmonella* and *Escherichia* were grown in batch A5W. On the other hand, "gas" was vigorously evolved in all cultures of the clostridia. This observation suggests that the clostridia may have decomposed other substances, besides formic acid, with the production of "gas".

Cl. perfringens, Cl. septicum, and *Cl. sporogenes* produced lactic acid. Their ability to yield lactic acid was in the order named. The yield from each organism was greatest in the medium in which the greatest consumption of sugar was found. Thus *Cl. perfringens* yielded 33% of lactic acid from 29.0 mM C_3; 53% was obtained from 57.4 mM C_3. *Cl. septicum* yielded 6% of lactic acid from 22.5 mM of C_3; and 27% from 49.7 mM C_3. *Cl. sporogenes* yielded 2% from 17.4 mM C_3, and only 9% from 47.6 mM C_3. The same trend is shown by the following data from *Escherichia coli* which was incubated at 37.5° in the same type of culture medium. The consumption of C_3 and the corresponding yield of lactic acid were as follows: 17.6 mM, 3%; 28.9 mM, 5%; 34.8 mM, 11%; 44.5 mM, 20%; 63 mM, 36%; 75.1 mM, 44%.

No conclusions can be reached from these data concerning the production of lactic acid by *Cl. tetani* and *Cl. botulinum,* since the consumption of sugar was small. These organisms removed lactic acid from the culture medium—a result so far not observed in this laboratory when intestinal organisms, streptococci, pneumococci, and staphylococci were grown anareobically over a short period of time.[21–25]

A striking feature of the metabolism of these clostridia is their ability to produce butyric acid. Measurable quantities of butyric acid have not so far been found by us in young cultures of other pathogenic bacteria.[21–25] Whether this acid was derived from sugar or from amino acids cannot, of course, be determined from the data. But it is significant that the quantity was small. The presence of butyl alcohol could not be demonstrated.[‡]

Our results differ greatly from those given in the literature. This is due, perhaps, to the long period of incubation, lasting from several days to several weeks, used by other workers. During the long incubation the organisms bring about many secondary changes, such as the decomposition of formic acid, the conversion of acetic acid to alcohol and other products, and the further metabolism of lactic acid. Our data indicate that the carbohydrate metabolism of *young, rapidly growing,* pathogenic clostridia is similar in many respects to that of many other rapidly growing pathogens.[21, 22, 24, 25]

A large part of the carbohydrate is converted either into lactic acid or it is further metabolized to yield approximately 2 molecules of formic acid, and 1 molecule each of acetic acid and ethyl alcohol.

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[22] Friedemann, T. E., Proc. Soc. Exp. Biol. and Med., 1939, **40**, 505.

[23] Friedemann, E. E., *J. Biol. Chem.,* 1939, **130**, 61.

[24] Friedemann, T. E., *J. Biol. Chem.,* 1939, **130**, 757.

[25] Friedemann, T. E., Proc. Soc. Exp. Biol. and Med., 1940, **43**, 148.

[‡] The distribution coefficient of the volatile acids derived from the oxidation of the alcohols by chromic acid was the same as that of acetic acid.[19]

13047

Complement-Fixation Tests on Rabbits with Brown-Pearce Carcinoma.*

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The recent paper by Cheever[1] on complement fixing antibodies in rabbits bearing Brown-Pearce tumors, observed by Kidd,[2] prompts the publication of a similar study carried out in the Spring of 1939 in which similarly positive results were obtained at first, but appeared questionable after the introduction of more rigid controls.

The tumor strain was provided by Dr. J. B. Murphy and Mr. Ernest Sturm. Rabbits were inoculated by intratesticular injection. Antigens were prepared and the complement fixation tests carried out as described by Kidd.[2a]

Twenty-five rabbits in all were tested after inoculation with tumor. In the early tests on some 14 rabbits, sera of 3 out of 6 rabbits in which tumors were rapidly developing with many metastases showed definite complement fixation, as compared with 10 negative normal sera used for control. Two other sera were doubtful and one negative. Of 5 animals with small tumors all were negative; and of 3 animals in which tumors indubitably grew and then regressed, all were negative. It may be of interest to note that in 2 of these latter animals kidney metastases appeared to have attained a size of from 3 to 6 mm or larger and subsequently regressed.

However, difficulties were encountered in attempts to repeat this experiment. A considerable capacity of some normal sera to fix complement was observed under these conditions, and it was noted that the tumor extracts used as antigen also varied markedly in their complement fixing properties. Prolonged centrifugation, filtration through a Mandler filter, or simply passage of time reduced this tendency to fix complement. Attempts to distinguish more clearly between the sera of the normal and tumor-bearing rabbits by altering the strength of the antigen by dilution were unsuccessful, since a dilution of antigen which eliminated all reactions with

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* This investigation was aided by a grant from the Charlton Research Fund of Tufts College Medical School.

[1] Cheever, F. S., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 517.

[2] Kidd, J. G., a. Proc. Soc. Exp. Biol. and Med., 1938, **38**, 292; b. *J. Exp. Med.*, 1940, **71**, 335, 351; c. *J. Bact.*, 1940, **39**, 349.

normal sera usually also obliterated reactions with the sera of tumor-bearing rabbits.

Thus it was evident that the differences to be observed were small, and a more precise type of experiment was adopted. Samples of serum were taken from all rabbits before inoculation with tumor. Frequent, usually weekly bleedings were made from time of inoculation until death from the tumor, and all the samples from any one animal were tested at the same time, thus eliminating most differences due to individual variations in sera and antigens.

Tests were carried out in this way on 12 rabbits. Of 8 rabbits with rapidly progressing, generalized tumors, tests on 3 were doubtful and on 5 negative, and of 4 rabbits with small tumors, all were negative. These animals yielded no certain evidence of the production of complement-fixing antibodies. In no case did we succeed in showing that a serum, negative before inoculation, became definitely positive after inoculation when compared against the same antigen in the same test.

Comment. It is perhaps of significance that in these experiments and those of Cheever, the positive or doubtful results were almost all in animals with rapidly progressing tumors, since it is especially in those animals in which the tumors were regressing that one might expect to find antibody formation, if the formation of antibodies conferred any degree of immunity to the tumor. As sera are diluted only 1-4 in the tests, and positive results were obtained in only 5 of 35 rabbits in Cheever's series, and 3 of 25 rabbits in ours, it is evident that the effects were so slight and infrequent as to be open to several interpretations. Kidd[2b] has reported a few sera reacting in higher dilutions.

Both Kidd and Cheever emphasize the analogy to the serologically active substance extracted from virus-induced rabbit papillomas. Against this would seem to be the strikingly weaker reactions in the rabbits bearing Brown-Pearce carcinomas as compared with those infected with papilloma virus, and also Cheever's observation that various antigenic extracts differ in their specificity when tested with the same positive sera. Unless it is assumed that the reactions are haphazard, it seems probable that a number of antigens are involved. This suggests the possibility that the reactions observed might be due to the production of antibodies to altered antigens of rabbit organs, resulting from pathological processes induced by the tumor, rather than to a single tumor or virus antigen. Kidd noted[2b] that this possibility could not be excluded, and Dmochowski[3] has shown that sera of rabbits immunized against unheated aqueous extracts of Brown-Pearce carcinoma give cross reactions with pus of rabbits, though not with aqueous extracts of normal organs.

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[3] Dmochowski, L., *Compt. rendue de la Soc. de Biol.*, 1938, **129**, 349.

However, if future experiments should conclusively demonstrate the presence of specific tumor antibodies, it would still be necessary to exclude the obvious likelihood (Dmochowski[3]) that the antigenic substance is part of the cellular elements of the tumor, before the virus analogy could logically be pressed. The specificity of tumors such as the one under consideration is comparable to the specificity of transplants in which genetic factors strongly influence the course of growth. These genetic differences must be reflected in chemical differences. Thus it would seem possible that genetic differences between the Brown-Pearce tumor and its host could exist and result in the formation of antibodies. Due to more rapid growth, such antibodies might be more readily formed by a tumor than a transplant.

13048

Rumen Synthesis of the Vitamin B Complex on Natural Rations.*†

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During the past several years considerable work demonstrating the synthesis of the vitamin B complex in the rumen of polygastric animals has been reported.[1-4] The above mentioned work showed conclusively the synthesis of the vitamin B complex in the rumen when the animal was fed a ration devoid or nearly so of the B complex. What the picture would be if the animal was fed a natural ration containing the members of the B complex has as yet received

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* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

† Thanks are extended to Lester Teply for assistance in the bacterial assays for nicotinic acid and to George Bahler for the biotin determinations.

[1] Bechdel, S. I., *et al.*, *J. Biol. Chem.*, 1928, **80**, 231.

[2] McElroy, L. W., and Goss, H., *Proc. Am. Soc. Biol. Chem.*, 1940, **34**, lxv; *J. Biol. Chem.*, 1939, **130**, 437.

[3] Wegner, M. I., *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 769.

[4] Johnson, P., *et al.*, *J. Dairy Sci.*, 1941, **24**, 57.

TABLE I.
Composition of the Grain Mixtures.

Trial No.	Basal grain mix,* lb	Linseed oil meal, lb	Urea, lb	% protein (N x 6.25)
1	80	—	—	11.3
2	78	—	2.0	17.1
3	64.5	15.5	0.6	17.1
4	50	29	1.2	23.3
5	37	41	2.0	31.1
6	74.5	—	5.5	31.2

*Consists of a 50-50 mixture of ground corn and oats to which 1% salt and 1% bone meal were added.

little attention.[5] The opportunity to investigate this question presented itself during a study of the problem of inorganic nitrogen utilization by ruminants. We had available for this study a heifer with a rumen fistula from which samples of the rumen ingesta could be removed at will. The object of the work reported in this paper was to determine whether adding urea, linseed oil meal or both to the grain mixture of a basal ration in varying proportions had any influence on the synthesis of the B complex in the rumen.

The Holstein heifer used weighed approximately 1000 pounds and had a rumen fistula equipped with a rubber plug which insured almost anaerobic conditions in the rumen and prevented leakage or loss of rumen contents. The basal ration fed consisted of 15 pounds of corn silage, 4 pounds of timothy hay, and 4 pounds of grain mixture (½ ground corn, ½ ground oats). The amount of corn silage and timothy hay fed remained constant throughout the duration of the experiment. The only variable was in the grain mixture to which urea or oil meal, or both were added as listed in Table I.

The animal was fed daily at 8 a.m. and 8 p.m. Samples were taken only after an equilibrium period on each individual grain ration of a week or more. All samples were obtained 6 hours after feeding (2 p.m.). The animal always consumed all of the ration within one hour after feeding. The sampling was done by hand in such a manner as to obtain a representative aliquot of the rumen material. Four to 5 kilos of the wet material were obtained at each sampling. The samples were taken on alternate days until about 20 kilos of the wet material (13 to 15% dry matter) had been obtained. The ingesta were stored in a dark-cold room at 5-10°C in crocks for 4 to 8 days, having first been treated with 95% ethanol so that the final concentration of alcohol was approximately 50%. Then the wet material was dried in a dark drying room (temperature 40-50°C) in shallow enamel lined pans for 24-40 hours. The dry material was next finely ground in a Wiley mill and stored in glass jars in the cold room until needed for assay. The assays were run on a composite of all samples taken for a particular trial. All of the above mentioned steps were taken in order to standardize the conditions with each ration that was tried.

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[5] Hunt, C. H., *et al.*, *J. Nutr.*, 1941, **21**, 85.

The methods of assay used were as follows: Thiamin, chick;[7] riboflavin, microbiological;[8] nicotinic acid, chemical[9] and microbiological;[10] pyridoxine, rat;[11] pantothenic acid, microbiological;[12] biotin, microbiological.[13]

Examination of Table II shows only a slight variation in the B complex content with the various grain mixtures fed. The greatest variation was in the protein or nitrogen content of the rations.

Table III lists the 6 components of the vitamin B complex assayed for in the rumen ingesta. As can be observed, nearly all the factors are higher in the ingesta than in the feed. Data are also

TABLE II.
B Complex Content of Rations and Ration Constituents Fed.
(Gamma per gram dry matter.)

Material assayed	Thiamin	Riboflavin	Pyridoxine HCl	Ca Pantothenate	Nicotinic acid		Biotin
					Chemical	Bacterial	
Basal grain mix	7-8.0	2.28	2-3	16.0	177	20.0	.091
Timothy Hay	2.0	4.86	3-4	10.0	190	23.0	.032
Corn Silage	<0.5	9.92	1-2	4.7	303	15.0	.080
Linseed oil meal	5-6.0	<0.3	5-6	23.0	156	43.0	.166
Trial 1 (calc. ration content)	3.3	5.68	2.5	10.2	225	19.0	.068
Trial 2 (calc. ration content)	3.3	5.68	2.5	10.2	225	19.0	.068
Trial 3 (calc. ration content)	3.2	5.40	3.0	11.6	220	20.5	.073
Trial 4 (calc. ration content)	3.1	5.23	3.5	13.0	215	22.0	.078
Trial 5 (calc. ration content)	3.0	5.10	3.8	14.0	211	23.1	.081
Trial 6 (calc. ration content)	3.25	5.62	2.5	10.2	224	19.0	.068
Synthetic Calf Ration	0	<0.4	<1.0	<3.4	60	<1.0	<.018

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[7] Arnold, A., and Elvehjem, C. A., *J. Nutr.*, 1938, **15**, 403.

[8] Snell, E. E., and Strong, F. M., *J. Ind. and Eng. Chem., Anal. Ed.*, 1939, **11**, 346.

[9] Melnick, D., and Field, H., Jr., *J. Biol. Chem.*, 1940, **134**, 1.

[10] Snell, E. E., and Wright, L. D., Reported to Federation of Am. Soc. for Exp. Biol., April 15-19, 1941 (Chicago).

[11] Conger, T. W., and Elvehjem, C. A., in press.

[12] Strong, F. M., *et al.*, Reported to Am. Chem. Soc. Div. Biol. Chem., 1940 (Detroit).

[13] Lampen, J. O., *et al.*, Reported to Div. of Biol. Chem. of Am. Chem. Soc., Detroit, Mich., Sept. 9-13, 1940, p. 21.

TABLE III.
B Complex Content of Rumen Contents.
(Gamma per gram dry matter.)

Material assay	Thiamin	Riboflavin	Pyridoxine HCl	Ca Pantothenate	Nicotinic acid		Biotin
					Chemical	Bacterial	
Heifer—Trial 1	4-5	24.6	4-5	26.6	530	50	.080
" " 2	2-3	26.7	8-9	31.0	568	56	.125
" " 3	4-5	22.7	7-8	29.2	466	63	—
" " 4	3.5-4.5	16.3	10.3	27.6	444	58	—
" " 5	4-5	11.6	8.5	31.2	438	68	—
" " 6	—	13.6	—	24.4	—	66	—
" " 6 (dried at pH 4.7)	—	12.9	—	8.0	—	—	—
Calf—Trial 1	4-5	29.0	3-4	33.0	520	54	.091
Calf—On synthetic ration	10-12	18.6	7.0	55.5	220	73	.087
Calf—On synthetic ration (plus 200 mg B_1 per day)	>20	26.5	11-12	82.5	187	100	.250

given in Table III on the effect of an acid pH during drying on the amounts of riboflavin and pantothenic acid in the ingesta. In addition, the question of variation in the vitamin content of the rumen material of 2 different animals receiving the same ration was investigated, and the results included in Table III.

From the data it can be seen that as the nitrogen level of the rations being fed was increased there was little change in the vitamin levels with the exception of the riboflavin values which decreased. No explanation for the decrease is readily apparent. The increase in the components of the B complex found in the ingesta compared to the rations fed are not absolute values. That is, a number of variables will affect the values obtained. Since the factors assayed for are water soluble, the concentration might be greater, the higher the moisture content of the samples taken. This is due to the fact that analyses are based on dry matter. All our samples were very uniform in moisture content from trial to trial. Another factor that would cause variations is the time of sampling after feeding since bacterial activity and leaching do influence the vitamin content.[5]

We feel that the increase in B vitamins in the ingesta as contrasted with the ration fed is due to a synthesis and not to a concentration effect. The first reason for this conclusion is that the vitamin increases in the ingesta over the ration are not all of the same magnitude. Further evidence is the decrease in flavin with an increase of protein in the ration while the other factors show little change. Lastly, since the B vitamins are water soluble a decrease due to leaching rather than an increase would be expected in the ingesta if no synthesis took place.

The values obtained from the rumen content when the basal ration alone was fed, as compared to the other rations used, gives a clearer picture of any changes produced since the variable of rumen digestion and absorption is the same in each case and is therefore eliminated.

The duplication of these results with another animal was tried. Fortunately, another Holstein calf (400 pounds) with a rumen fistula was available. Trial 1 was repeated using this animal and the results obtained show that the figures are of the same magnitude as those found in the heifer.

Since our last report[3] a new method for the determination of nicotinic acid became available and we are therefore including two sets of figures for this substance. We feel that the bacterial method gives a more accurate estimation of the biological activity of the rumen ingesta for this factor.

For comparison, values previously reported[3] by feeding a "synthetic" ration to a calf are also included.

Treating the sample of rumen ingesta at a pH 4.5-5.0 did not enhance the flavin value as was reported by McElroy and Goss.[6] In fact, the acid had a destructive action on the pantothenic acid. However, the possibility exists that the differences are due to the types of rations used.

Summary. Six members of the vitamin B complex in the rumen ingesta of a heifer fed a ration composed of natural feeds were determined. In most cases higher values were found in the rumen ingesta than in the ration fed. With the exception of flavin, variation of the amount of urea or protein in the grain mixture of the ration had little if any effect on the vitamin content of the ingesta.

13049 P

Non-Specific Precipitation of Sulfathiazole Azo-Conjugates by Immune Sera; Inhibition and Complement-Fixation.

Samuel C. Bukantz and Theodore J. Abernethy. (Introduced by Michael Heidelberger.)

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A case of acquired sulfathiazole sensitivity exhibiting a specific response to administration of single doses of sulfathiazole has recently been described.[1] Similar observations have been made on a patient sensitized to sodium sulfapyridine.[2] The specificity of the response prompted a search for the presence of antibodies specific for the sulfonamide grouping. Attempts to detect such antibodies to pure sulfonamide drug antigens have failed;[3] test antigens were therefore prepared by coupling diazotized sulfathiazole with crystalline horse serum albumin, horse serum globulin, and human serum globulin.[4] Similar azo antigens have already been employed in studies of human sulfonamide sensitivity with negative results[5, 6] although the development of specific antibodies by the rabbit immunized with sulfonamide azo proteins has been described.[3, 7]

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[6] McElroy, L. W., and Goss, H., *J. Nutr.*, 1940, **20**, 541.

The sulfathiazole azo proteins precipitated with some supposedly normal and several unrelated immune sera. Similar precipitation of azo proteins, in high concentration, was encountered by Landsteiner and Van der Scheer,[8] who diluted their antigens to eliminate the non-specific precipitation. Resorcinol-disazosulfathiazole (ResDST) was also synthesized[9] to determine whether exclusion of protein from the molecule would eliminate non-specific precipitation. The unrelated sera,* however, also precipitated with ResDST. The amount of precipitation depended upon the proportion in which serum and ResDST were mixed and inhibition zones were encountered with excess of antibody or antigen (Table I). With certain serum-dye ratios exposure to 37°C resulted in solution of the precipitate, the amount of precipitate in other mixtures changing in character from dispersed floccules to a cohesive disc. Precipitations of other azo proteins and horse antipneumococcal sera have already

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[1] Abernethy, T. J., Bukantz, S. C., and Minor, J., Presented at the Society of Clinical Investigation, Atlantic City, May 5, 1941.

[2] Davidson, A. D., and Bullowa, J. G. M., *New Eng. J. Med.*, 1940, **22**, 811.

[3] *Vide* 2 and Wedum, A. G., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 218, for bibliography.

[4] a. Landsteiner, K., and Lampl, H., *Z. Immunitats Forsch.*, 1917, **26**, 293; b. Heidelberger, M., Kendall, F. E., and Soo Hoo, C. M., *J. Exp. Med.*, 1933, **58**, 137.

[5] Schlesinger, E. R., and Mitchell, W. L., Jr., *Am. J. Dis. Child.*, 1938, **56**, 1256.

[6] Wedum, A. G., *J. Bact.* (abstr.), 1941, **41**, 64.

[7] Mingoia, Q., Rocha Lima, and Silva, M., *Boll. d'inst. sieroterap. milanese*, 1940, **19**, 101.

[8] Landsteiner, K., and Van der Scheer, J., *J. Exp. Med.*, 1927, **45**, 1050.

[9] Landsteiner, K., and Van der Scheer, J., *J. Exp. Med.*, 1932, **56**, 399.

* Rabbit anti-horse serum albumin; anti-horse serum globulin, anti-sheep cell, antipneumococcal 3 (Lederle), and anti-egg-albumin (kindly supplied by Dr. Michael Heidelberger); horse antipneumococcal 1 (Lederle) and horse anti-egg-albumin (kindly supplied by Dr. A. W. Pappenheimer, Jr.). Normal horse and rabbit sera and 2 horse antitoxins failed to precipitate with these antigens.

TABLE I.

Effect of Varying Antigen-antibody Ratios on the Amount of Precipitate Formed by Rabbit Antipneumococcal 3 Serum and Resorcinoldisazosulfathiazole.

0.2 cc of the desired serum dilution delivered, by pipet, into 0.2 cc of antigen. Readings after 1 hour at room temperature. Saline controls were negative.

Serum* dilution	Molar concentration of resorcinoldisazosulfathiazole				
	M/50	M/500	M/2,500	M/25,000	M/50,000
Undiluted	++++	+++	+±	±	±
1-5	0	+++±	++±	+	±
1-20	0	++±	++	+	±
1-80	0	+±	++	+±	±
1-320	0	±	+±	+	±
1-1280	0	0	+	+	±

*Rabbit antipneumococcal 3 serum No. 473H370h (Lederle), 9,000 units per cc (Kindly supplied by Dr. W. G. Malcolm).

been described;[10, 11, 12] rabbit antipneumococcal sera, however, failed to precipitate.[11]

It seemed of interest to determine whether the homologous pure sulfonamide drugs or their neutral azo components would inhibit the precipitation between sulfathiazole azo protein and the non-azo-specific antipneumococcal rabbit serum. M/10 solutions of the pure sulfonamide drugs can be obtained only with the soluble sodium salts which are highly alkaline in this concentration, M/10 sodium sulfapyridine having a greater titratable alkalinity than M/10 sodium sulfathiazole. The precipitation of sulfathiazole azo protein by antipneumococcal rabbit serum was completely inhibited by a saline solution equal in titratable alkalinity to M/10 sodium sulfapyridine and M/10 sodium sulfathiazole caused no greater inhibition than an equally alkaline saline solution. Simple azo components of haptens are more active inhibitors than pure haptens.[13] The inhibitory activity of a simple azo compound of sulfathiazole, ResDST, was also investigated. 0.3 cc of M/50 ResDST completely inhibited the precipitation between 0.2 cc of antipneumococcal 3 rabbit serum and 0.5 cc of 1 to 500 sulfathiazole azo crystalline horse serum albumin. This observation suggests that inhibition depends upon the chemical identity of the antigenic components of the reaction and does not necessarily indicate the presence, within the precipitating serum, of specific antibodies to the hapten.[13]

In complement-fixation studies, 2 units of complement were completely fixed by the resorcinol conjugate in the presence of anti-

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[10] Woolf, B., Marrack, J. R., and Downie, A. W., *J. Soc. Chem. Ind.*, 1936, **55**, 156.

[11] Goebel, W. F., and Hotchkiss, R. D., *J. Exp. Med.*, 1937, **66**, 191.

[12] Goebel, W. F., *J. Exp. Med.*, 1936, **64**, 29.

[13] Landsteiner, K., *Bioch. Z.*, 1920, **104**, 285.

pneumococcal horse or rabbit sera. Complement fixation by atoxyl azo antigens and homologous antisera have already been described.[14]

The authors gratefully acknowledge the valuable assistance given during the course of this study by Dr. M. Heidelberger, of Columbia University, Dr. Hugo Bauer, of the National Institute of Health, Drs. O. T. Avery and K. Landsteiner, of the Rockefeller Institute for Medical Research, and Dr. E. A. Kabat, of Cornell University.

13050

Excretion of Sulfathiazole in Tears.

Jacob Turkell and Seymour F. Wilhelm. (Introduced by E. H. Fishberg.)

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Seven cases of conjunctivitis occurring during the administration of sulfathiazole have been observed at the Beth Israel Hospital during the last 5 months. Quantitative determinations of the sulfathiazole concentration in the tears were made in 4 of these. Similar quantitative studies were carried out in 10 patients who were taking sulfathiazole but had no evidence of conjunctivitis (Table I). Blood sulfathiazole determinations were made in all cases at the time that the lachrymal secretion was collected.

Technic. The eyelids were first carefully cleansed with ether to rid them of sebaceous material. Tears were stimulated by the gentle application to the inner canthus of a cotton swab moistened with ether. The lachrymal secretion was then collected at the outer canthus by a fine capillary pipette. Controls had shown that this procedure did not cause any conjunctival irritation. Since sulfathiazole secretion in sweat had been demonstrated by the authors, care was exercised not to collect any tears which had overflowed on to the cheek.

The total amount of lachrymal secretion obtained from both eyes varied from 0.4 cc to 0.9 cc, and, in each case, 1.2 cc of 4 N HCl was added to the entire measured amount. This was then diluted with distilled water to a volume of 25 cc. Ten cc of this was used as a blank control. Another 10 cc was diazotized using N (1-naphthyl)

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[14] Klopstock, A., and Selter, G. E., *Z. Immunitats. Forsch.*, 1928, **55**, 450; **57**, 174.

TABLE I.

		Therapy		Conc. of drug, mg%		
No.	Case No.	Total No. g given orally	No. days administered	Blood	Tears	Comment
1	128647	8	1	2.6	.40	No conjunctivitis
2	128701	12	1	4	.57	” ”
3	128448	6	1	3.4	.35	” ”
		12	2	3.6	.30	” ”
4	128843	4	1	2.8	.56	” ”
		12	3	2.3	.64	” ”
5	128277	8	1.5	4.8	.40	” ”
		23	4	3.8	.40	” ”
6	128767	8	2	4.4	.50	” ”
		23	6	3.4	.98	” ”
7	128864	4	1	2.8	.64	” ”
		12	3	3.6	.66	” ”
		22	6	3.4	.70	” ”
8	128917	4	1	2.9	.30	” ”
		16	4	2.8	.10	” ”
		26	7	2.6	.75	” ”
9	128473	28	9	9.8	.90	” ”
10	128839	29	8	5.0	.50	” ”
11	128136 1st course	4	1	3.6	.30	” ”
		21	4	3	.80	Mild conjunctivitis, bilateral, palpebral and bulbar injection.
		24	5	3.4	.60	Severe conjunctivitis, bilateral.
12	128758 1st course	10	1.5	8.5	.75	Mild conjunctivitis, bilateral, palpebral and bulbar injection.
13	127632 2nd course	4	1	1.7	.21	No conjunctivitis.
		12	3	4.2	.75	Mild conjunctivitis, left eye. Moderate conjunctivitis, right eye. Triangular patch of bulbar injection.
14	125546 4th course	6	2	3.8	.66	Moderate conjunctivitis of right eye only.

ethylene-diamine dihydrochloride as a coupler.[1] Readings were then made on a photo-electric colorimeter.

Analysis of Results. Sulfathiazole was found in the tears of the 14 patients examined in concentrations varying from 0.1 mg % to 0.98 mg %. The concentration tended to rise with the continued administration of the drug. However, no relationship was established between the blood and the tear levels. For example, of 6 patients who showed a rise in tear concentration, only 2 (Cases 9 and 11) revealed a corresponding rise in the blood level. Three patients (Cases 4, 8 and 9) failed to show any consistent blood elevation and one (Case 6) showed a definite decline. A constant tear level was demonstrated in Case 5 despite a fall in the blood concentration. These findings indicate that the amount of drug secreted in the tears is not proportional to the blood concentration, but depends perhaps on other factors such as the secretory function of the lachrymal gland. The relationship between the blood and tear concentration also does not appear to be proportional to the water content of these 2 fluids and in this respect sulfathiazole differs from sulfanilamide.[2]

The sulfathiazole concentration in the tears reached 0.6 mg % or more in all cases of conjunctivitis. On the other hand, in 6 of the 10 cases without conjunctivitis, the concentration was 0.56 or more. Obviously, therefore, the conjunctival inflammation cannot be ascribed to an increased concentration of sulfathiazole in the tears. The eyes of 5 patients not receiving sulfathiazole were irrigated for 15 minutes of every hour for a period of 16 hours with solutions of 0.3 mg %, 0.8 mg %, 2 mg %, 5 mg %, and 10 mg % of sulfathiazole respectively. Conjunctivitis did not appear in any of these patients.

Discussion. The conjunctivitis occurring during the administration of sulfathiazole is, in all likelihood, a reaction peculiar to this drug. Although sulfanilamide has been found in tears in greater concentration than sulfathiazole,[3, 4] conjunctivitis following its administration has not been observed. In like manner, conjunctivitis due to sulfapyridine has not been seen. Of the 7 cases of sulfathiazole conjunctivitis observed by the authors, 4 had no eye manifestations during the first course of therapy. The conjunctivitis in these cases appeared during the second or during a subsequent course, suggesting that this reaction is due to drug sensitization in susceptible individuals.

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[1] Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, **128**, 537.

[2] Marshall, E. K., Jr., Emerson, K., Jr., and Cutting, W. C., *J. Pharm. and Exp. Therap.*, 1937, **61**, 196.

[3] Bellows, J. G., and Chinn, H., *J. A. M. A.*, 1939, **112**, 2023.

[4] Venco, L., *Ann. di Ottal. e Clin. Ocul.*, 1939, **67**, 30.

The eye nearest the window and therefore more exposed to sunlight, was in one instance the only one involved (Case 12), and in another (Case 11) it was more severely inflamed.

Haviland and Long[5] reported 6 instances of conjunctivitis in 78 patients receiving sulfathiazole. In 31 consecutive patients treated by the authors on the Urological Surgical Service, 5 instances of conjunctivitis were observed. This greater incidence may be due to the fact that in many of our cases more than one course of therapy was given. No serious sequelae were seen, and the conjunctivitis disappeared in from 1 to 4 days, following cessation of therapy. It has been accepted[5] that this complication is an indication for the interruption of the sulfathiazole therapy. However, in one of our cases the administration of the drug was continued despite the conjunctivitis since no other signs of toxicity were present. The eyes were treated with drops of .25% zinc sulphate solution and after 4 days the conjunctivitis disappeared completely despite the uninterrupted administration of sulfathiazole. However, in view of the frequent relationship of conjunctivitis to other more severe toxic manifestations it is safer to discontinue the drug immediately if conjunctivitis appears.

Summary. The tears of 14 patients receiving sulfathiazole were quantitatively analyzed. Sulfathiazole was found in all the cases. The concentrations varied from 0.1 mg % to 0.98 mg %.

Four patients suffering from sulfathiazole conjunctivitis showed a tear concentration of 0.6 mg %, or more. On the other hand, 6 of 10 patients without conjunctivitis had a tear concentration of 0.56 mg %.

A single case of conjunctivitis was observed in which the eye inflammation disappeared without sequelae despite the uninterrupted administration of sulfathiazole.

Among 31 consecutive patients receiving sulfathiazole, 5 developed conjunctivitis. In 4 of these the conjunctivitis appeared during a second or a subsequent course of therapy.

Conclusions. 1. The amount of sulfathiazole secreted in the tears is not proportional to the blood sulfathiazole concentration.

2. The concentration of sulfathiazole in the tears does not of itself appear to be the cause of the conjunctivitis.

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[5] Haviland, W. J., and Long, P. H., *Bull. Johns Hopkins Hosp.*, 1940, **66**, 313.

13051

Virus of Spontaneous Encephalomyelitis Found in Intestines of Normal Wild Gray Mice.

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Practically all of more than 300 normal, adult, albino mice (Rockefeller Institute, Swiss, and other strains) thus far tested, harbored in their intestines the virus of spontaneous encephalomyelitis (Theiler's disease; "mouse poliomyelitis").[1, 2] The experimental and the spontaneous forms of this disease have been shown to be closely similar to human and experimental (monkey) poliomyelitis; moreover, there is a resemblance in many properties of the viruses causing the respective maladies.[1, 2] A recent study[3] of albino mice revealed no essential difference between the lesions of experimental and spontaneous Theiler's disease and those[4] of the experimental infection induced by the Lansing strain[5] of human poliomyelitis.

Albino mice have occurred among gray mice under domestication and have then been inbred. They are used chiefly as stock animals in biological laboratories, while the animal from which they are derived, the wild gray mouse, is not only a commensal of man but in its predatory existence it can contaminate man's food with its excreta. The almost universal presence of a virus, having so much in common with that of human poliomyelitis, in the intestines and feces[1, 2] of the albino mice, is therefore of less significance to an epidemiologist than would be the finding of Theiler's virus in the intestines of wild gray mice. Hence, a search was undertaken for Theiler's virus in the intestines of apparently normal, wild gray house-mice, and those most readily at hand were the ones trapped in one of the animal houses of the Rockefeller Institute.

At the outset it should be noted that these gray mice are as susceptible to Theiler's virus, given intracerebrally, as are their albino

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[1] Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 434; *J. Exp. Med.*, 1940, **72**, 113.

[2] Theiler, M., and Gard, S., *J. Exp. Med.*, 1940, **72**, 79.

[3] Olitsky, P. K., and Schlesinger, R. W., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 79.

[4] Lillie, R. D., and Armstrong, C., *Pub. Health Rep.*, 1940, **55**, 718.

[5] Armstrong, C., *Pub. Health Rep.*, 1939, **54**, 1719.

TABLE I.
Theiler's Virus in Intestines of Adult Wild Gray Mice.

Exp. No.	No. of mice used	Pooled materials used	Paralysis produced	Remarks
1	3	12% suspension entire intestinal tract	0/8*	
		10% susp. of brain	0/8	
2	2	10% susp. of contents only of entire intestinal tract	7/8	Brain to brain 2nd passage with 10^{-1} to 10^{-6} suspensions, 84/95. Similarly, 3rd passage with 10^{-1} suspension, 10/10.
		10% susp. of brain	0/7	
3	3	10% intest. contents	7/10	
		10% susp. of brain	0/10	
4	5	10% intest. contents	4/14	
		10% susp. of brain	0/6	
5	5	10% intest. contents	1/14	
		10% susp. of brain	0/6	
6	4	10% intest. contents	2/14	
		10% susp. of brain	0/6	

*The numerator represents the number showing paralysis and the denominator the number of albino mice injected.

derivatives. The encephalomyelitis so induced in both strains is indistinguishable on clinical, pathological, and immunological grounds; furthermore, the experimental disease in gray mice is transmissible and can be repassaged to albino mice.

The methods used have already been described.[1] Table I shows the results obtained in 6 experiments in each of which the intestinal material and, in parallel, the brains of from 2 to 5 gray mice were pooled.

The outcome of 5 of the 6 experiments clearly points to the presence of the encephalomyelitic virus in the intestines of the apparently normal, gray mice trapped at the Institute. Further studies revealed that the intestinal virus deriving from the gray mice is not different from that obtained from albino strains in clinical, pathological, and immunological (cross-immunity) properties and also in its non-pathogenicity in *rhesus* monkeys.[6] As in albino mice, no virus was detected in the brains of the normal gray mice; in the former, virus was found in the brain only when they exhibited clinically apparent Theiler's disease.[1, 2]

Discussion. Based on lesser proportion of positive results of pools, it would appear that not as many apparently normal gray mice harbor Theiler's virus in their intestines as do albino ones.[1, 2] (*Cf.* Theiler[7].)

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[6] Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 339.

Although rigid quarantine and isolation are practiced in the animal house, no such control can be applied to predatory, wild gray mice. Hence the possibility arises that they were contaminated with Theiler's virus in an endemic focus populated by virus-carrying albino mice. This has not as yet been proved a fact; meanwhile, 5 experiments in which were examined 7 house and 3 field gray mice, all trapped at a distance from the laboratory, failed to show virus in their intestines or brains. Dr. Max Theiler reports, in addition, that virus was not detected in a much larger series of gray mice trapped away from the Institute.[7]

Conclusions. (1) Wild gray house-mice are as susceptible to intracerebral introduction of Theiler's virus as are albino mice. (2) Apparently normal gray mice trapped in an animal house in which almost all adult albino mice are carriers of Theiler's virus, reveal this virus in their intestines and not in their brains. (3) The virus is indistinguishable from that obtained from the intestines of normal albino mice. (4) The number of gray mice shown to be carriers of Theiler's virus, while considerable, appears to be lower than that of carriers found among the albino strain.

Preliminary tests to the present time have failed to indicate that the virus is demonstrable in gray house and field mice trapped at a distance from the laboratory. This subject is still under investigation.

13052

Correlation of Histological Differentiation with Beginning of Function of Developing Thyroid Gland of Frog.

Aubrey Gorbman and Herbert M. Evans.

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The capacities for removal of inorganic iodine from the blood stream and for storage of such iodine in organic form in relatively high concentration are well known properties of the adult thyroid gland. The recent use of "tagged" radioactive iodine has provided the tool for the striking, graphic demonstration of these properties of the thyroid.[1] The accumulation of administred radio-iodine in thyroid tissue was revealed by these workers by placing sections of tissue in contact with X-ray films.

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[7] Theiler, M., Studies on Poliomyelitis, De Lamar Lecture, in press.

Using this method for revealing accumulated iodine, an experiment was devised to show at what stage of differentiation of the thyroid gland the ability for storage of iodine could first be demonstrated. Sixty-three tadpoles of the frog, *Hyla regilla,* were placed in a glass container in 800 cc of tap water. To this water was added an amount of radio-iodine,* as the sodium salt, which had a total activity of 150 microcuries. The preparation used has a half-life of 8 days. The mass involved was less than 0.1 mg.

The tadpoles, which were of all sizes from the just-hatched 7.0 mm larva to the 38 mm larva with well developed hind limbs (5.0 mm long) were kept in the radio-active water for 2 days. Most of the animals were fixed in formalin and serially sectioned. The lower jaws of the remaining tadpoles were dried on glass slides. Radio-autographs were made of the whole lower jaws, and of the serial sections by allowing the glass slides bearing them to remain in contact with a sensitive "no-screen" X-ray film. The serial sections were then stained in hematoxylin-eosin for matching with the radio-autographs. The staining properties of the tissues were slightly, but not seriously, impaired by the drying and handling incident to making the radio-autographs.

No evidence of storage of iodine by the thyroid could be found in larvae having a total length of less than 10 mm. Division of the thyroid lobes occurred at about 7 mm total length and beginning formation of follicles at about 9.0 mm. Stokes[2] has provided a complete description of the normal development of the thyroid in this frog. In the 10.0 mm larva (body length 3.0 mm) the first faint radio-autographs (Fig. 1) were produced by the 2 lobes of thyroid tissue. At this stage each thyroid lobe consisted of about 3 to 6 follicles with very small, if any, lumen. Each follicle was from 10μ to 15μ in diameter and was circumscribed, in cross section by about 4 to 6 cells. In the 12.0 mm larva (4.0 mm body length), although differentiation had not proceeded much farther (each lobe 6 to 7 follicles, 18μ to 28μ in diameter, 6 to 9 cells in cross section) the radio-autograph was somewhat darker. As the thyroid increased

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[1] Hamilton, J. G., Soley, M. H., and Eichorn, K. B., *University of Calif. Publ. Pharmacol.*, 1940, **1**, 339.

* The writers are indebted to the Radiation Laboratory of the University of California, and especially to Dr. J. G. Hamilton for the radio-active iodine used in this work, and to Mr. M. Murayama for assistance with photomicrography.

[2] Stokes, M., Thesis, University of California, 1941 (unpublished).

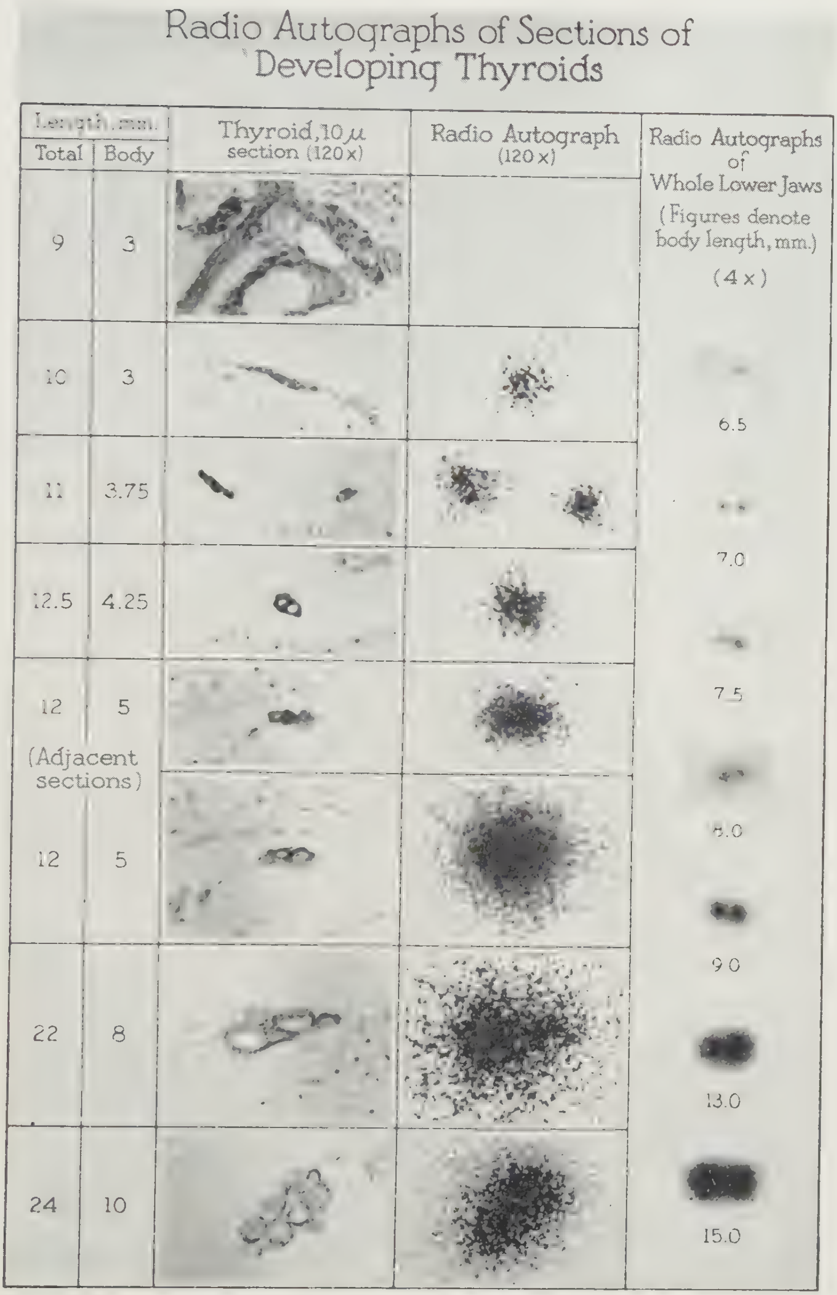
Radio Autographs of Sections of Developing Thyroids
Length, mm.
Total
Body
Thyroid, 10 µ section (120 x)
Radio Autograph (120 x)
Radio Autographs of Whole Lower Jaws
(Figures denote body length, mm.)
(4 x)
9
3
10
3
11
3.75
12.5
4.25
12
5
(Adjacent sections)
12
5
22
8
24
10
6.5
7.0
7.5
8.0
9.0
13.0
15.0

Fig. 1.

in size, and colloid increased in amount, the sections produced progressively blacker records. It is interesting to note in the figures of the thyroid of the 5.0 mm body length tadpole that of the adjacent sections, the one containing more colloid contained also much more radio-activity, revealed by a striking difference in the radio-autographs.

It is clear, therefore, that in this frog function by the thyroid can be demonstrated very early after formation of follicles within each of the 2 primitive, still yolk-laden, lobes. It should be remembered that the iodine in the serial sections which produced the radio-autographs had remained in the thin section even after immersion in aqueous and alcoholic solutions, in alcohol-ether, and xylene. The iodine is, therefore, quite likely in an organic linkage, probably as thyroglobulin.

13053 P

Origin and Differentiation of Epithelium of Urinogenital Sinus in the Opossum.

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Previous results have shown[1, 2] that administration of estrogens to young opossums during early pouch life produces extreme hyperplasia and cornification of the lining of the urinogenital sinus and structures associated developmentally with the sinus. The related structures are the *neck* and *trigone* region of the bladder, and the *sinus horns* (dorso-lateral diverticula of the sinus which received the gonoducts and subsequently contribute extensively to the *lateral vaginal canals*). The sinus epithelium undergoes intense squamous metaplasia with continuous shedding of keratinized layers after estrogen treatment, closely resembling the adult vagina at estrus. Similar results are reported by Moore.[3]

Where other epithelia of the urinogenital tract join the sinus the former do not respond, and the transition is abrupt. The response was, therefore, considered specific for sinus epithelium. In 20-day

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[1] Burns, R. K., Jr., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 270.

[2] Burns, R. K., Jr., *J. Morph.*, 1939, **65**, 497.

[3] Moore, C. R., *Physiol. Zool.*, 1941, **14**, 1.

young such transitions occur where the gonoducts join the sinus horns, and at the borders of the trigone; at the external meatus, however, the sinus lining continues indistinguishably into the epidermis, and at all levels of the sinus the metaplastic mucosa shows the same close resemblance to skin that characterizes the adult mammalian vagina.

In younger specimens, treated from 10 to 14 days, the reaction is less advanced and a progression is clearly seen. Hyperplasia is most extreme and cornification most advanced in the neighborhood of the meatus and in the lower sinus. Both conditions diminish toward the bladder. In the *sinus horns* and in the *trigone,* stratified squamous epithelium from the sinus can be seen actively eroding and replacing the primitive linings of these areas. Appearances clearly indicate an invasion of this tissue from below, with the process greatly accelerated by hormone action.

Since the sharply limited and specific nature of the cornification response indicates a common origin of the tissues involved from the sinus; and since the characteristic histological features are evoked in the order mentioned, the original derivation of the cornifying epithelium from the ectodermal portion of the cloaca is suggested. Early development decidedly favors this view: the ectodermal cloaca or uro-proctodeum is rather deep in the opossum[4] and a core of ectodermal epithelium extends toward both anal and urinogenital divisions of the cloaca, as the cloacal membrane breaks down approximately 12 hours before birth. The urethral plate is likewise a potential source.[5] The sinus is very shallow at this time and entering tissue would at once lie at a level just below the sinus horns. Shortly after birth the linings of sinus and ectodermal cloaca appear continuous and identical; and pigmented, keratinized cells (like those of the epitrichium) already occur in the lower sinus. At the base of the bladder 3 kinds of epithelium may be distinguished: (1) a thick, many-layered tissue lines the sinus as far as the bladder neck; (2) the trigone area is covered by simple, cuboidal epithelium continuous with that of the mesonephric duct; (3) the bladder proper and the sinus horns are lined by an irregular epithelium of so-called transitional type. The last-mentioned appears to represent the primitive cloacal endoderm. In older experimental animals, as we have seen, the linings of sinus horns and trigone are replaced by stratified squamous (cornifying) epithelium from the sinus; and the primitive cloacal endoderm appears to be restricted eventually to the bladder. Elongation of the canal plus active ingrowth readily explain the presence of the invading tissue at the horn level in older stages (and later still in the lateral vaginal canals).

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[4] McCrady, Edw., Jr., *J. Morph.*, 1940, **66**, 131.

[5] Barnstein, N. J., and Mossman, H. W., *Anat. Rec.*, 1938, **72**, 67.

Recently Zuckerman,[6] reviewing an extensive literature, has reached the same conclusions with regard to a common origin from the urinogenital sinus for tissues sensitive to estrogens; and Raynaud's results on the cat[7] deserve particular mention since he reports cornification of urethra and trigone. Zuckerman also raises, but does not decide the question of ectodermal contribution to the sinus. This is an old problem, never conclusively attacked, but now fairly clearly answered in the opossum.

Summary and Conclusions. 1. Those epithelia in the urinogenital tract of the opossum which respond to estrogens by squamous metaplasia and cornification, have a common origin from the lining of the urinogenital sinus. 2. The sinus epithelium itself is apparently derived at an early stage of development from the ectodermal cloaca or uro-proctodeum, and perhaps in part from the urethral plate. 3. The primitive cloacal endoderm is eventually restricted to the bladder proper. 4. A common ectodermal origin best explains the histological and physiological likenesses of the tissues involved, and the occurrence of stratified squamous epithelium in the lower urinogenital tract of mammals generally. Early development in higher mammals needs reinvestigation with respect to this point. 5. The use of hormones (particularly estrogens) in the immature organism is a valuable method of precociously differentiating tissues not so readily delimited in normal development of the urinogenital tract.

13054

Decomposition of Urea by Proteus.

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At the Third International Congress for Microbiology St. John-Brooks and Rhodes[1] suggested a simplification of the taxonomy of the Proteus genus. On the basis of maltose fermentation and indol production 3 species were set up in the genus, *indologenes (vulgaris)*, *morganii*, and *anindologenes (mirabilis)*. *Proteus americanus* and *Proteus ammoniæ* were considered variants of *anindologenes*. *Proteus hydrophilus*, *Proteus pseudovaleriei*, and *Proteus icthyosmius* were excluded from the genus on the basis of their ability to ferment mannitol.

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[6] Zuckerman, S., *Biol. Rev.*, 1940, **15**, 231.

[7] Raynaud, A., *C. R. Soc. Biol.*, 1937, **126**, 215.

[1] St. John-Brooks, R., and Rhodes, M., *Third International Congress for Microbiology, Report of Proceedings*, 1939, 167.

The decomposition of urea with formation of ammonia is one of the major criteria for the determination of the Proteus group (Bergey).[2] This phenomenon, however has received relatively little attention, particularly when recent additions have been made to this genus. From a review of the literature it appears that the action on urea of *Proteus morganii*, *Proteus hydrophilus*, *Proteus icthyosmius*, and *Proteus bombycis* is unknown.

Investigators, with few exceptions, have used sterile urine as a culture medium to test urea decomposition by Proteus. Complications arising from the use of such a variable medium are obvious. Moltke[3] and Yacob[4] employed a medium consisting of 2% urea in 0.85% saline. These investigators point out that substances in the inoculum initiate growth and we have found that serial transplants in their medium fail to grow. After several attempts a medium of known composition has been devised, in which small inocula grow and in which serial transplants can be made.

Materials and Methods. The medium finally employed contained 2% urea (Merck), 0.01% yeast extract (Difco), and M/15 primary and secondary phosphate buffers (Sorensen) in distilled water to give a final pH of 6.8. Sterilization was accomplished by filtration through Berkefeld filters (Type N). The test medium was inoculated from 24-hour agar cultures and incubated at 37°C. Nessler's reagent was employed to determine the presence of ammonia by adding a loopful of the culture to a drop of the reagent on porcelain plates. In order to follow the pH changes, a portion of the medium was made up with phenol red in a concentration of 1-100,000/ml, and another portion with thymol blue in a concentration of 1-50,000/ml, thus covering the pH range from 6.8 to 9.8. A portion of the medium without urea, but containing yeast extract, buffer, and indicator, controlled pH changes in the basic medium while another portion with yeast extract and buffer alone controlled the production of ammonia from the basic medium.

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[2] *Bergey's Manual of Determinative Bacteriology*, 1939, Fifth Edition, Williams and Wilkins Co., Baltimore.

[3] Moltke, O., *Contributions to the Characterization and Systemic Classification of Bac. proteus vulgaris* (Hauser), 1927, Levin and Munksgaard, Copenhagen.

[4] Yacob, M., *Indian J. Med. Research*, 1932, **19**, 787.

A total of 160 known Proteus strains were tested, including *Proteus vulgaris, Proteus mirabilis, Proteus morganii,* the X strains, and one strain each of *Proteus americanus, Proteus ammoniæ, Proteus icthyosmius, Proteus bombycis,* 2 strains of *Proteus pseudovaleriei,* and 4 strains of *Proteus hydrophilus.* In addition 31 cultures isolated in other laboratories and tentatively classed as Proteus strains were also included. Tests for ammonia were made and pH changes observed at 4, 8, 24, and 48 hours after inoculation. Cultures negative after 48 hours were held an additional 72 hours.

The results are summarized in Table I. All members of the genus except *Proteus hydrophilus, Proteus icthyosmius,* and *Proteus bombycis* were capable of attacking urea. At 4 hours color reactions of yellow, yellow orange, and orange were obtained with the Nessler test for *Proteus vulgaris, Proteus mirabilis, Proteus ammoniæ, Proteus pseudovaleriei, Proteus americanus,* and with one exception the X strains. The exception, which will be discussed in a subsequent paper, was a non-motile variant of a motile culture. The pH reactions varied from 6.9 to 7.2 at 4 hours and from 7.2 to 8.0 at 8 hours, whereas at 24 hours all were practically the same, 9.2 to 9.4. In 2 days the limit of the thymol blue indicator was reached with a majority of these strains. Variation in the pH reaction might result from differences in the size of the inoculum or from slightly different rates of activity of the various strains.

Although the *Proteus morganii* strains were capable of attacking urea, their activity was markedly slower. At 8 hours every strain gave a positive Nessler test, but 9 strains did not raise the pH in this time. Two days were required for all *morganii* strains to give a pH reaction of 8.0 to 8.2, thus definitely indicating a much slower rate of urease production.

The 31 cultures tentatively classified as Proteus, the 4 strains of *Proteus hydrophilus,* and the single strains of *Proteus icthyosmius** and *Proteus bombycis** neither gave a reaction for ammonia nor changed the pH reaction over a period of 5 days.

To determine the efficiency of urease production in a chemically defined medium, nicotinic acid and pantothenic acid were substituted for the yeast extract since these substances, present in yeast extract, have been recently described as growth factors for Proteus.[5, 6] Representative Proteus strains, inoculated so that no initial turbidity

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* American Type Culture Collection.

[5] Fildes, P., *British J. Exp. Path.*, 1938, **19**, 239.

[6] Pelczar, M. J., and Porter, J. R., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 151.

TABLE I.
Urea Decomposition by Proteus. Control Medium pH 6.8 No Reaction in Any of Cultures at 120 hr. Test Medium pH 6.8.

		NH_3 (No indicator in medium)				
Cultures	No. of strains	Hr 4	8	24	48	96 and 120
Pr. vulgaris	28	Y-YO	YO-O	O		
Pr. mirabilis	81	Y-YO	YO-O	O		
X strains	24	Y-YO	YO-O	O		
X strains	1	—	—	—	—	—
Pr. pseudovaleriei	2	YO	O			
Pr. americanus	1	YO	O			
Pr. ammoniæ	1	Y	O			
Pr. morganii	16	Y	Y	YO	O	
Pr. hydrophilus	4	—	—	—	—	—
Pr. icthyosmius	1	—	—	—	—	—
Pr. bombycis	1	—	—	—	—	—
Unknown strains	31	—	—	—	—	—
		pH (Indicator in medium)				
Pr. vulgaris	28	6.9-7.2	7.2-8.0	9.2-9.4	9.6-9.8	
Pr. mirabilis	81	6.9-7.2	7.2-8.0	9.2-9.4	9.6-9.8	
X strains	24	6.9-7.2	7.2-8.0	9.2-9.4	9.6-9.8	
X strains	1	—	—	—	—	—
Pr. pseudovaleriei	2	6.9	7.4	9.4	9.6	
Pr. americanus	1	7.1	7.8	9.4	9.6	
Pr. ammoniæ	1	6.9	7.4	9.4	9.6	
Pr. morganii	16	6.8	6.8-7.0	7.1-7.3	8.0-8.2	8.2-8.8
Pr. hydrophilus	4	—	—	—	—	—
Pr. ichthyosmius	1	—	—	—	—	—
Pr. bombycis	1	—	—	—	—	—
Unknown strains	31	—	—	—	—	—

Y, weak reaction; YO, moderate reaction; O, strong reaction; —, no reaction.

was present, gave weak to moderate positive reactions in 48 to 72 hours. Urea decomposition was possible in such a synthetic medium but it was apparent that the medium was not nearly as effective as the yeast extract medium.

Discussion. Yeast extract serves to provide the essential growth factors for Proteus. All cultures gave uniform growth in the control medium without producing ammonia or changing the pH in 5 days.

The most recent addition to the Proteus group has been *Proteus morganii* on the basis of Rauss' investigations.[7] The production of the enzyme urease by this organism in our estimation is further justification for its classification under Proteus. Its rate of activity, however, distinctly differs from that of the other species and appears to be constant for all strains of *morganii.*

St. John-Brooks and Rhodes do not consider *hydrophilus* and

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[7] Rauss, K. F., *J. Path. and Bact.*, 1936, **42**, 183.

icthyosmius members of the genus Proteus because of their mannitol fermenting property and we would also exclude these strains from the genus because of their inability to attack urea. A similar conclusion can be drawn with regard to *bombycis* since it also failed to attack urea. The 2 strains of *Proteus pseudovaleriei** which decomposed urea did not conform to the description of Bergey and of St. John-Brooks and Rhodes. One strain appeared to be *Proteus vulgaris* and the other *Proteus mirabilis.* On the other hand, we do not agree with St. John-Brooks and Rhodes that the names *vulgaris* and *mirabilis* should be changed.

13055

Selective Action of Sulfanilyl-Guanidine on Different Salmonella Types and Its Practical Importance.*

S. BORNSTEIN AND L. STRAUSS. (Introduced by E. H. Fishberg.)
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Marshall and coworkers[1] have produced a new sulfa-compound, sulfanilyl-guanidine, and studied its bacteriostatic effect. The solubility of this substance in water at 37.5°C is 220 mg %, its absorption from the intestines comparatively poor. These properties make the drug particularly fit for use in intestinal infections.

In order to study the action of sulfanilyl-guanidine[†] on different organisms of the Salmonella group, we tested 74 types listed in the latest edition of the Kauffmann-White schema[2] and 13 types which were described since. Fifteen strains of *E. coli* isolated from human feces, urine and blood, 4 strains of Shigella (2 Flexner, 1 Sonne, 1 Shiga type) and a strain of Aerobacter were also tested.

For preliminary information on differences in susceptibility the agar strip method was used. Strips 1 cm wide were cut out of agar plates and filled in with a suspension of 1% sulfanilyl-guanidine in agar. The cultures were streaked out across the inlaid strips.

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* Aided by a grant from the Ella Sachs Plotz Foundation.

[1] Marshall, E. K., Jr., Bratton, A. C., White, H. K., and Litchfield, J. T., Jr., *Bull. Johns Hopkins Hosp.*, 1940, **67**, 163.

[†] We received the sulfanilyl-guanidine through the courtesy of the research department of E. R. Squibb & Sons.

[2] *J. Hyg.*, 1934, **34**, 33; Report of the Salmonella Subcommittee of the Nomenclature Committee of the International Association of Microbiologists, June, 1939.

Of all the Salmonella organisms tested, only the typhoid bacillus *(S. typhi), S. paratyphi A* and *S. cholerae suis* showed any marked degree of inhibition. Very slight inhibitory action was noted on *S. paratyphi C, S. abortus equi, S. sendai, S. gallinarum, S. muenster* and *S. newington.*

While Aerobacter was resistant and all 4 dysentery strains were strongly inhibited, the 15 strains of *E. coli* showed individual differences. In Salmonella organisms, however, no marked differences between strains of the same type were noticed, when 2 strains of *S. paratyphi A,* 7 strains of *S. cholerae suis,* 6 strains of *S. typhi* and 10 strains each of *S. paratyphi B* and *S. typhi murium* were tested.

Test tube experiments were then carried out to determine more accurately the bacteriostatic action of sulfanilyl-guanidine on representative Salmonella types and control organisms, 30 in all. Test tubes containing 4 cc of beef infusion broth with the drug in decreasing concentrations were inoculated with 0.1 cc of 24-hour broth cultures in serial dilutions, and incubated at 37.5°C for 24 hours. The results confirmed the findings by the agar strip method.

The differences between individual strains of *E. coli* were of minor degree in the test tube experiment, especially so with small inocula. The 2 extreme results are shown in Table II.

It was concluded that sulfanilyl-guanidine which has a marked bacteriostatic action on *E. coli* and *S. typhi,* and acts very strongly on Shigella, is practically without effect on other Salmonella types

TABLE I.
Bacteriostatic Effect of Sulfanilyl-guanidine. Medium: Beef-infusion Broth.

Strain	Inoculum No. organisms	Conc. of sulfanilyl-guanidine, mg%					
		200	100	50	25	10	—
S. paratyphi A No. C2	10	—	—	—	—	+	+
S. paratyphi B No. C3	10	+	+	+	+	+	+
S. typhi murium No. C9	7	+	+	+	+	+	+
S. choleræ suis No. 36	10	—	—	—	—	—	+
S. thompson No. C39	2	—	+	+	+	+	+
S. newport No. 50	18	+	+	+	+	+	+
S. typhi H-901 No. C57	10	—	—	+	+	+	+
S. gallinarum No. C74	13	—	+	+	+	+	+
S. enteritidis No. C64	10	+	+	+	+	+	+
E. coli No. 3648	10	—	—	—	+	+	+
Shigella Flexner (D.I.)	30	—	—	—	—	—	+

+, macroscopic growth after 24 hr; —, culture tube clear after 24 hr.

TABLE II.
Difference in Susceptibility of Strains of *E. coli* Against Sulfanilyl-guanidine.

	No. organisms in inoculum	Mg% Sulfanilyl-guanidine in beef infusion broth					
		200	100	50	25	10	—
E. coli No. 8/41	800	—	+	+	+	+	+
	80	—	—	—	+	+	+
	8	—	—	—	—	+	+
E. coli No. 248/41	600	+	+	+	+	+	+
	60	—	+	+	+	+	+
	6	—	—	—	+	+	+

except *S. paratyphi A* and *S. cholerae suis.* Neither mutual antigenic components nor any other mutual characteristic can be cited as an explanation for the fact that just these types are more susceptible to the drug than others. *S. gallinarum* and *S. paratyphi C,* antigenically related to *S. typhi* and *S. cholerae suis,* respectively, showed weak susceptibility to the drug while *S. thompson,* also closely related to *S. cholerae suis,* was completely resistant.

Our findings seem to indicate that sulfanilyl-guanidine treatment of infections with Salmonellas other than the susceptible types may even be harmful. As a result of the selective action of the drug the pathogenic organisms may flourish while the coli flora is suppressed. A small number of animal experiments, carried out so far, support this opinion.

The 24-hour growth of an agar slant was washed off with 3 cc of tap water, pipetted onto a piece of bread and fed to a mouse of about 20 g after the animal was fasting over night. Three groups of mice were thus infected with *S. cholerae suis, S. typhi murium,* and *S. enteritidis,* 8 with each organism. From the second day the mice were fed 5 g per day Rockland mouse diet and water only. Four mice of each series received 1% by weight of sulfanilyl-guanidine well admixed to the solid food. After 3 weeks the drug treatment was discontinued in 2 of the surviving mice and carried on with half doses in the 2 others.

All the mice infected with *S. enteritidis* died within 3 to 8 days. Of the mice infected with *S. typhi murium* only one out of 4 non-treated animals died, but all 4 mice treated with the drug died within 7 to 11 days.

On the other hand, with *S. cholerae suis,* all the non-treated animals died within 16 days, while the treated ones all survived for more than 3 weeks. Only those whose treatment was discontinued died thereafter. Whether these animals were reinfected from their

TABLE III.
Effect of Sulfanilyl-guanidine Treatment on Salmonella Infections in Mice.

Strain	Not treated				Treated			
S. choleræ suis No. C36	6	6	7	16	31*	25*	S†	S†
S. typhi murium No. C9	11	S	S	S	7	7	9	11
S. enteritidis No. 3612	3	4	4	6	3	3	6	8

The numbers indicate the days after which the mice succumbed.
S: survived more than 40 days.
*: drug discontinued after 21 days.
†: drug continued with half the doses after 21 days.

fur, or whether the organisms survived within their bodies remains a matter of doubt.

Our contention that sulfanilyl-guanidine, though promising for the treatment of infections with *S. paratyphi A* and *S. cholerae suis,* is ineffective in other Salmonella infections, is further supported by the few observations available thus far on Salmonella infections in children treated with the drug.

Marshall and coworkers state in their recent paper[3] in which they report on successful treatment of acute bacillary dysentery with sulfanilyl-guanidine: "Two other infants, age 5 and 8 months, respectively, were treated on the third and fourth day of acute gastroenteritis, without beneficial effect. A variety of *S. enteritidis* was cultured from the stools in one of these children, and the Morgan bacillus and later a variety of *S. enteritidis* from the stools of the other."

Two children who after acute infections with *S. oranienburg* and *S. newport* continued to excrete the organisms in their stools, were treated with the drug in this hospital. Doses of 0.3 g per kg body weight for 8 and 12 days respectively failed to interrupt the series of positive stool cultures. Another child had an acute infection with *S. cholerae suis* and continued to excrete the organism in the stools. Upon our suggestion he was given the drug by Dr. S. Silvers and immediately after institution of the treatment the stool cultures became negative.

In conclusion, it can be said from *in vitro* and *in vivo* experiments, that sulfanilyl-guanidine, a drug which is known to be effective in the treatment of bacillary dysentery, is also effective against *S. cholerae suis* and, to some degree against *S. paratyphi A,* but ineffective against other organisms of the Salmonella group. In fact, in such cases the drug may even be harmful.

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[3] Marshall, E. K., Jr., Bratton, A. C., Edwards, L. B., and Walker, E., *Bull. Johns Hopkins Hosp.*, 1941, **68**, 94.

13056

Comparative Effect of Vitamin K and Whole Blood on Prothrombin Deficiency of Newborn Infant.

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The etiologic importance of a plasma prothrombin deficiency in the bleeding tendencies of obstructive jaundice, biliary fistula, and severe liver damage has been receiving prominent attention.

In 1937, it was first shown[1] that the bleeding in hemorrhagic disease of the newborn is a manifestation of prothrombin lack. Recent investigators[2-14] have reported a physiological depression of the plasma prothrombin during the first few days of life in the majority of infants. The infant's prothrombin is essentially normal at birth but falls to 20-50% of normal within the next few days and returns to its original level by the fifth to seventh day. Chart 1 illustrates the curves obtained in 3 normal infants. When this depression is extreme, infants may have a hemorrhagic tendency (melena, hematemesis, etc.).[5-9]

It should be noted that the various one-stage methods used for prothrombin determinations[15-17] measure not only prothrombin con-

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[1] Brinkhous, K. M., Smith, H. P., and Warner, E. D., *Am. J. M. Sc.*, 1937, **193**, 475.

[2] Waddell, W. W., Guerry, D., Bray, W. E., and Kelley, O. R., Proc. Soc. Exp. Biol. and Med., 1939, **40**, 432.

[3] Owen, C. A., Hoffman, G. R., Ziffren, S. E., and Smith, H. P., Proc. Soc. Exp. Biol. and Med., 1939, **41**, 181.

[4] Quick, A. J., and Grossman, A. M., Proc. Soc. Exp. Biol. and Med., 1939, **41**, 227.

[5] Waddell, W. W., and Guerry, D., *J. A. M. A.*, 1939, **112**, 2259.

[6] Nygaard, K. K., *Acta obstet. et gynec. Scandinav.*, 1939, **19**, 361.

[7] Dam, H., Tage-Hansen, E., and Plum, P., *Ugesk. f. laeger*, 1939, **101**, 896.

[8] Dam, H., Tage-Hansen, E., and Plum, P., *Lancet*, 1939, **1**, 1157.

[9] Quick, A. J., and Grossman, A. M., *Am. J. Med. Sc.*, 1940, **199**, 1.

[10] Bray, W. E., and Kelley, O. R., *Am. J. Clin. Path.*, 1940, **10**, 154.

[11] Kato, K., and Poncher, H. G., *J. A. M. A.*, 1940, **114**, 749.

[12] Macpherson, A. I. S., McCallum, E., and Haultain, W. F. T., *Brit. M. J.*, 1940, **1**, 839.

[13] Fitzgerald, J. E., and Webster, A., *Am. J. Obstet. and Gyn.*, 1940, **40**, 413.

[14] Kove, S., and Siegel, H., *J. Pediat.*, 1940, **17**, 448.

[15] Quick, A. J., *Am. J. Physiol.*, 1936, **114**, 282.

[16] Ziffren, S. E., Owen, C. A., Hoffman, G. R., and Smith, H. P., Proc. Soc. Exp. Biol. and Med., 1939, **40**, 595.

[17] Kato, K., *Am. J. Clin. Path.*, 1940, **10**, 147.

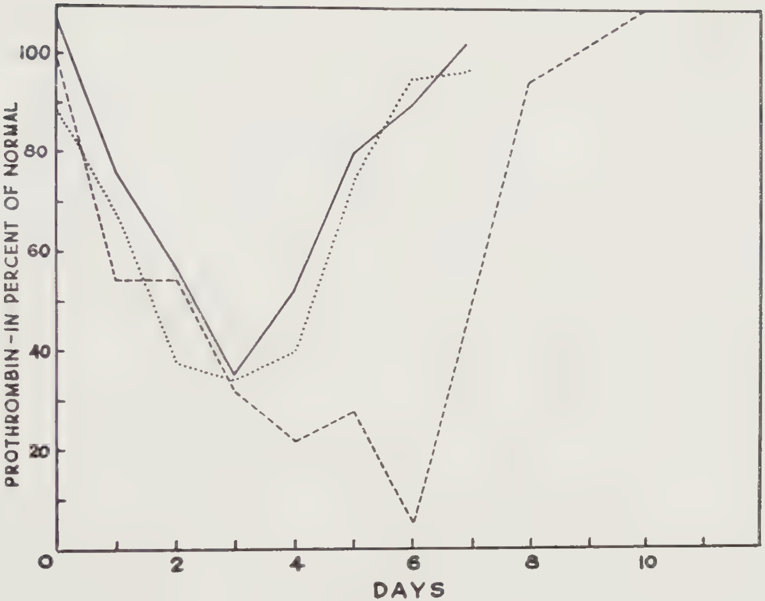

CHART 1.
Daily prothrombin levels in 3 normal newborn infants.

centration but also the rate of prothrombin conversion to thrombin. Inasmuch as this conversion rate is variable and can at times compensate for a low prothrombin,[3, 16, 18, 19] the methods employed furnish a more reliable index of a possible bleeding tendency than does the more complex 2-stage test[20] which measures only prothrombin concentration.

The time-honored treatment of hemorrhagic disease of the newborn is the administration of whole blood, either intravenously, intramuscularly or subcutaneously. The prophylactic and curative rôle of vitamin K in this disease was first reported in 1939[2] and is now well established. The purpose of this study was to compare the effect of vitamin K and of maternal whole blood on the prothrombin time of newborn infants.

Methods. Twenty-three healthy infants, 3 or 4 days old and weighing 2650 to 4900 g, were used. At this age the plasma prothrombin approaches its lowest level and remains depressed for at least one day.

Aqueous solutions of 4-amino-2-methyl-naphthol, a synthetic form of vitamin K, (Synkamin; Parke, Davis and Company), were

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[18] Schønheyder, F., *Am. J. Physiol.*, 1938, **123**, 751.

[19] Brinkhous, K. M., *Med.*, 1940, **19**, 329.

[20] Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667.

administered orally, intramuscularly and intravenously, in doses of one milligram. This preparation may produce vomiting when given by mouth.

Maternal whole blood was given subcutaneously and intramuscularly in 15 cc doses and the same amount was administered intravenously after the addition of 2 cc of 2.5% sodium citrate solution. Each of 2 babies was given 7 cc of human serum by the intravenous route. The serum was obtained from whole blood which was allowed to clot spontaneously and then stand for 12 hours, when less than 1% of the original prothrombin remained.

Determinations of prothrombin levels were performed by a microadaptation of the "Bedside" test.[16] Capillary blood, obtained by heel puncture, was mixed with thromboplastin in a 10:1 ratio, on a clean glass slide. As in the "Bedside" test, prothrombin is recorded in percent of normal:

$$\frac{\text{clotting time of normal}}{\text{clotting time of patient}} \times 100.$$

Results. It will be noted in the charts that even when the age factor is constant there is considerable variation in the initial prothrombin level. As controls, several additional prothrombin determinations were made on the blood of 5 untreated infants with initial readings ranging from 40 to 65%. Invariably the values remained essentially constant during a 10-15-hour observation. We have assumed, therefore, that the initial reading for any infant established the prothrombin level for that baby, for the succeeding several hours.

Administration of synthetic vitamin K to 6 infants, regardless of route, resulted in a rapid rise in the prothrombin level (Chart 2). In every case the prothrombin values were at least 50% of normal within 3-4 hours and 70% in 6 hours.

Fifteen cubic centimeters of whole blood, when injected intravenously into 4 babies, raised the prothrombin level approximately to the point which would have been expected from the addition of the prothrombin contained in the added blood (Chart 3). This slight prothrombin elevation appeared shortly after the blood was introduced. Comparable quantitative increases in the plasma prothrombin following transfusions have been noted in adults.[19] In 2 infants whose prothrombin levels were 10% of normal at the time of transfusion, the values did not rise out of the "danger zone" (less than 40%) within the period of observation, but there was no bleeding. Vitamin K was injected intramuscularly into one of these

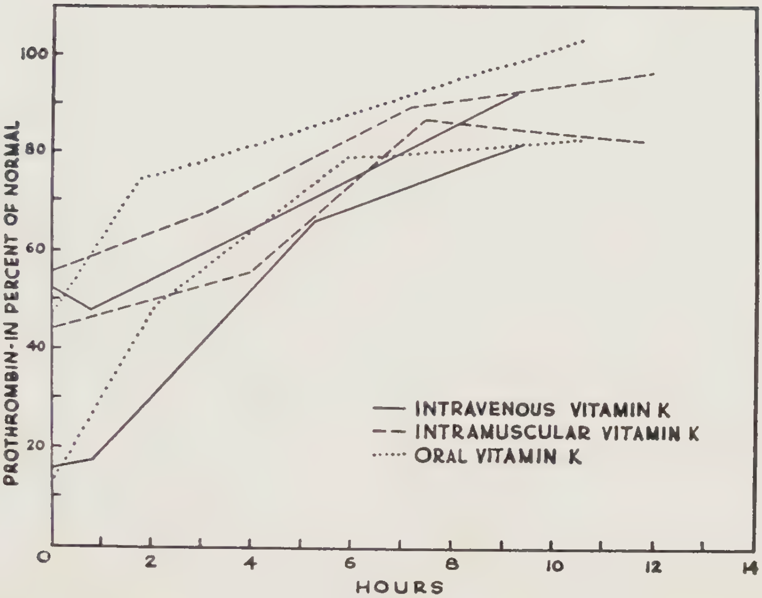

CHART 2.
Prothrombin levels in 6 infants after the administration of vitamin K.

2 infants 4 hours after the blood had been given; the prothrombin rose from 20% to 80% of normal in 8 hours (Chart 3).

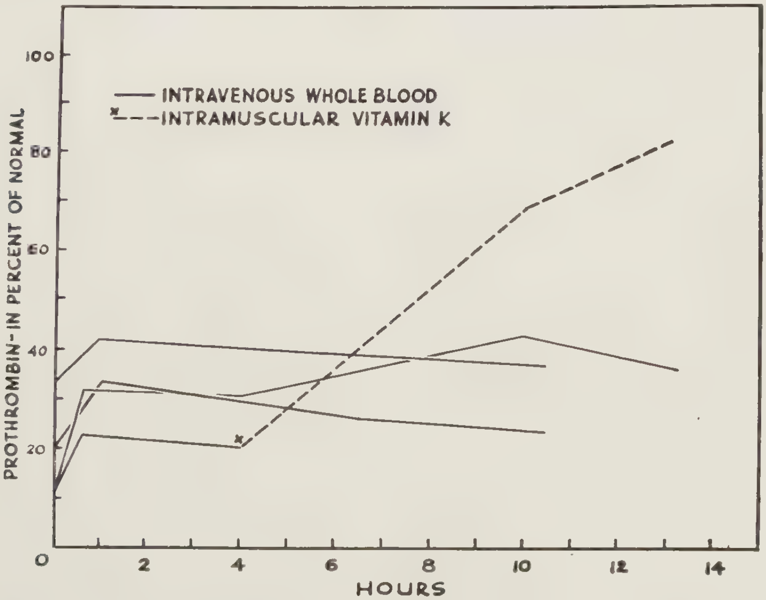

CHART 3.
Prothrombin levels in 4 infants after the injection of whole blood intravenously.

We were unable to demonstrate any prothrombin elevation when whole blood was injected subcutaneously or intramuscularly into 6 infants (Chart 4 shows representative cases).

That no "stimulating" action is present in the serum portion of blood is indicated by the lack of prothrombin elevation following intravenous injection of prothrombin-free serum into 2 infants (Chart 4).

Discussion. Vitamin K, given orally or parenterally, causes a rapid rise in the plasma prothrombin level of newborn infants with hypoprothrombinemia whether there is a hemorrhagic tendency or no clinical manifestation of the depressed prothrombin level. Whole blood has a slight additive effect when injected intravenously. Satisfactory treatment with adequate blood transfusions (30-50 cc) has the disadvantage of requiring such specialized technical proficiency that it is frequently not available when needed. Moreover, some time is lost by the need for cross-matching. Injections of blood, other than intravenously, have no effect on the clotting time within 12-15 hours, as measured by plasma prothrombin levels.

Summary. Observations made upon a group of 3- and 4-day-old infants, who showed the expected hypoprothrombinemia, indicate

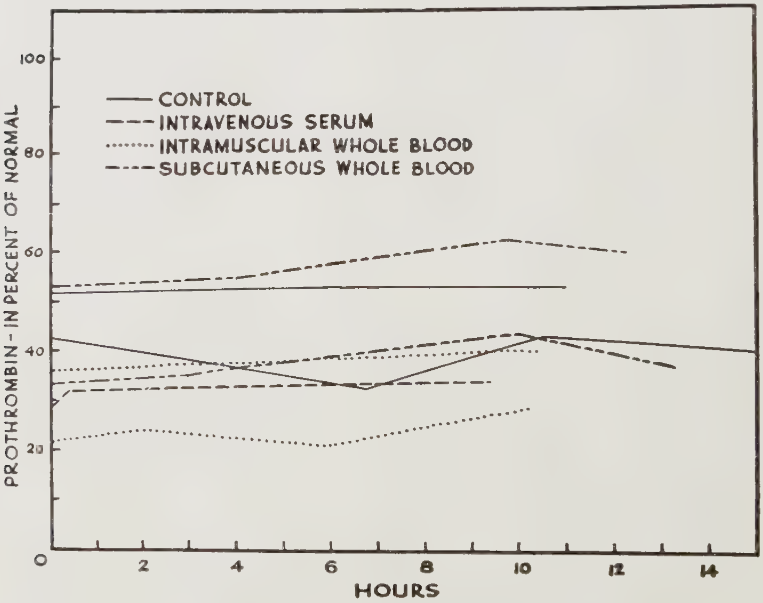

CHART 4.

Comparative effect of whole blood administered intramuscularly and subcutaneously, and of human serum injected intravenously, on prothrombin levels of normal infants.

that maternal whole blood, regardless of the mode of administration, is not as effective as vitamin K in raising the plasma prothrombin level. Because of the known relationships of reduced prothrombin levels to hemorrhagic disease of the newborn, it is suggested that treatment with vitamin K, which produces very rapid and significant elevations of the prothrombin, will prove much more effective than injections of maternal blood.

13057

Differential Cell Counts of Ant. Lobe of Pituitary Glands of Rats Showing Diabetic Traits.

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Recent reports[1-5] on studies of a strain of rats with diabetic traits have presented evidence of hyperfunction of anterior lobe of the pituitary gland. The purpose of this investigation was to study the anterior lobe of the pituitary gland of this strain by the method of cell enumeration. No critical cytological study was done.

Material and Methods. The pituitary glands of 15 sexually mature male rats of the "diabetic" strain, and of an equal number of a "non-diabetic" strain matched in sex, age and weight, were studied. The average age of the "diabetic" rats was 233 days; of the controls, 212 days. The average weight of the "diabetic" rats was 451 g; of the controls, 347 g. The glands were not weighed. They were fixed in Bouin's solution modified according to Gomori,[6] whose method for fixing pancreatic islets was followed. The whole gland was embedded in paraffin at 57°C. Serial sections were cut in the coronal plane at 5 microns and all sections were mounted. The sections were stained by Mallory's Acid Fuchsin-Aniline Blue method as modified by Crooke and Russell.[7] We found that better results were

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[1] Cole, Versa V., and Harned, B. K., *Endocrinology*, 1938, **23**, 318.

[2] Harned, B. K., and Cole, Versa V., *Endocrinology*, 1939, **25**, 689.

[3] Cole, Versa V., and Harned, B. K., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 738.

[4] Harned, Ben K., and Cole, Versa V., *Science*, 1940, **92**, 361.

[5] Cole, Versa V., Harned, Ben K., and Keeler, Clyde E., *Endocrinology*, 1941, **28**, 25.

[6] Gomori, G., *Am. J. Path.*, 1939, **15**, 498.

[7] Turner, O. A., *J. Lab. and Clin. Med.*, 1939, **24**, 997.

obtained if the sections were stained in the acid fuchsin and the aniline blue solutions kept at 57°C. We did not stain with hematoxylin.

The counting technic was briefly as follows: A Whipple micrometer was placed in a No. 10 ocular. Using this with a 1.25 mm oil immersion objective and a mechanical stage every fifth section was studied. Differential counts were made in every fifth field in the transverse and vertical diameters. The average number of cells counted in each gland of the "diabetic" rats was 4,009 cells; in that of the controls 3,251 cells. Although our technic differed from that of other investigators in that we limited our count to cells in transverse and vertical diameters we believe statistical analysis shows that the fields counted were representative of the whole gland and that for purposes of comparison of the cell percentages the method is satisfactory.

TABLE I.
Differential Cell Counts of Anterior Lobe of the Pituitary Gland.

''Diabetic'' rats (males)					Controls (males)				
		% of cells					% of cells		
Age in days	Body wt	Eosinophiles	Basophiles	Chromophobes	Age in days	Body wt	Eosinophiles	Basophiles	Chromophobes
398	565	34.3	14.3	51.4	367	350	35.2	10.0	54.8
380	520	35.9	11.2	52.9	367	360	52.4	8.9	38.7
264	420	65.4	3.9	30.7	217	270	42.6	13.5	43.9
245	470	46.9	9.1	44.0	205	315	52.0	10.6	37.4
206	430	48.5	20.8	30.7	199	320	50.3	5.7	44.0
215	360	56.4	7.9	35.7	197	320	58.1	8.3	33.6
210	430	52.1	55.9	31.9	207	350	46.3	10.0	43.6
210	540	38.4	27.0	34.6	208	380	43.0	18.4	38.6
210	390	45.4	9.3	45.3	208	390	44.7	17.0	38.3
180	520	44.1	16.8	39.1	183	415	34.5	27.4	38.1
180	450	39.9	26.4	33.7	177	350	35.9	24.2	39.9
178	480	47.9	18.6	33.5	178	325	41.4	12.8	45.8
144	400	45.7	14.7	39.6	151	350	27.8	38.5	33.6
178	420	41.0	13.5	45.5	151	300	53.2	9.1	37.7
144	380	49.3	10.7	40.0	151	360	55.8	10.4	33.9

TABLE II.
Statistical Analysis.

Cell types	Min.	Max.	Median	Mean	Stand. dev.	Coeff. of variation	Standard error
		A. ''Diabetic'' Rats.					
Eosinophile	34.3	65.4	45.7	46.1	8.1	17.5	2.2
Basophile	3.9	27.0	14.3	14.7	7.5	51.4	2.0
Chromophobe	30.6	52.9	39.1	39.2	7.2	18.4	1.9
			B. Controls.				
Eosinophile	27.8	58.1	43.7	44.8	9.4	20.1	2.1
Basophile	5.7	38.5	10.6	15.0	7.9	52.5	2.1
Chromophobe	33.6	54.8	38.6	40.1	5.7	14.3	1.5

Conclusion. There is no significant difference between the percentages of cell types in the anterior lobe of the pituitary glands of 15 sexually mature male rats with diabetic traits and those of controls.

13058

Specific Neutralization of Cotton Rat Strains of Poliomyelitis Virus.*

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Paralysis has been produced in the eastern cotton rat by injecting combinations of poliomyelitis virus and "enteric toxin filtrate."[1] (Virus strains isolated were: Flexner's M. V., Flexner's Philadelphia, and SK New Haven.) In addition, Armstrong's and Jungeblut's strains have been carried 30 and 20 generations in rats, respectively. Flexner's M.V. rat-adapted strain was found to be specifically neutralized by poliomyelitis antiserums.[2] This paper is a report on neutralization experiments with Flexner's Philadelphia, Armstrong's Lansing and Jungeblut's strains.

The set-up of the experiments was similar to those previously reported.[2] There were 5 groups of animals, 30 in each group. Those in Group A were injected with a mixture of the antiserums used—equal amounts of serum and saline. Group B received 4% virus in saline mixed with an equal amount of serum termed "early horse serum" obtained from horses which were found lacking in virucidal antibodies for poliomyelitis virus.[3] Group C received 4% virus in saline and an equal amount of serum termed "convalescent horse serum" obtained from the same horses a few years later after they had been injected with virus.[3] Group D received 4% virus in saline plus an equal amount of pooled human convalescent poliomyelitis serum, and Group E, 4% virus plus saline to make a 2% suspen-

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* Sponsored by The National Foundation for Infantile Paralysis, Inc.

[1] Toomey, John A., and Takacs, William S., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 364.

[2] Toomey, John A., and Takacs, William S., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 319.

[3] Toomey, John A., *Am. J. Dis. Child.*, 1937, **52**, 1492.

sion. The amounts injected were: 0.06 cc intranasally, 0.06 cc intracerebrally, and 0.5 cc subcutaneously.

In neutralizing experiments, there are often insufficient negative and positive controls. In these experiments, there were as many in the negative and positive control groups as there were in the virus and serum injected.

Results. A. *Flexner's Philadelphia Strain.* All positive controls (Group E) died and only 1 of 30 negative controls (Group A). All animals of Group B, which received virus plus "early horse serum," died. Four animals (13.3%) in Group C, protected with convalescent horse serum" and 5 (16.6%) in Group D, protected with pooled convalescent human serum, died.

Conclusion. Bearing in mind the percentage of experimental error, the number of natural deaths that would occur and those that would follow immediately or within 24 hours after injection, etc., one could conclude that specificity had been demonstrated and that this strain of rat virus was truly an acclimated one from Flexner's Philadelphia virus.

B. *Armstrong's Lansing Strain.* All the positive controls (Group E) died; all negative controls (Group A) lived. All animals injected with "early horse serum" and virus (Group B) died. Two (6.6%) of Group C, the "convalescent horse serum" protected and 4 (13.3%) of Group D, given pooled convalescent human serum, died.

Conclusion. Bearing in mind the fact that 100% of the positive controls died and none of the negative as contrasted with mortality rates of 6.6% and 13.3% in the protected groups, it can be concluded that here also we dealt with a specific type of poliomyelitis virus acclimated to the cotton rat.

C. *Jungeblut's Strain.* Three specimens of mouse brain virus were received from Jungeblut. This strain was adapted to cotton rats and mice by Jungeblut and Sanders from strain SK New Haven, Generation XI, (Trask and Paul). This virus was easily transferred to other mice and to cotton rats. The latter were used for these experiments. In the doses used by us, the animals usually died of an overwhelming disease within 3 or 4 days after injection; in many instances they had respiratory muscle paralysis. If the animals lived longer than 4 days, they usually became paralyzed in the hind quarters, although some also had respiratory involvement.

Our results with virus received 7/18/40 were opposite to those had with other adapted strains (Flexner's M.V., Flexner's Philadelphia or Armstrong's Lansing). The animals in the negative control Group A lived. Every animal in the other 4 groups died. There was no prolongation of life between the time of the injection and death in any group, which would denote some specific protection.

The same experiment with the second lot of material obtained from Jungeblut 2/23/41 was repeated in 150 rats and the results were the same. A third experiment with a third lot of Jungeblut's material received 4/9/41 injected into a similar number of animals again gave the same results. No evidence nor tendency toward specific neutralization was demonstrated when cotton rats were used as the test animals.

Jungeblut's original adaptation came from Paul and Trask's SK strain (11th transfer generation). We obtained from Trask some of the 13th generation of the same strain. We had difficulty in adapting it to rats. Paralysis would often appear for 3 generations and then the strain would be lost. It was finally combined with "enteric toxin filtrate" and transferred to the eighth generation, but its virulence was nothing like that of the other acclimated strains. The animals injected with our adapted Trask virus and which developed paralysis and died did not die in the same manner as those which had been injected with Jungeblut's acclimated virus obtained from the same source. The incubation period was longer and the course of the disease less abrupt. It is possible that we were not experimenting with the same virulent quotient.

Does poliomyelitis virus change its character as it becomes modified to the mouse; does the virulence of the original virus become enhanced to the point where all animals die even when minute doses are used for the injection; do we deal with a separate virus entirely? These questions cannot be answered by our experiments.

Conclusion. It can only be stated (1) that the specimens sent to us by Jungeblut and Sanders were not neutralized by specific convalescent serums as were other poliomyelitis virus strains and (2) that our present adaptation of Trask's virus in rats does not produce clinical symptoms similar to those which follow injections of Jungeblut and Sanders strain.

13059 P

Maintenance of Pregnancy in the Hypophysectomized Rat.‡

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Evidence is accumulating that a factor closely associated with the lactogenic principle of the anterior pituitary may be responsible for functional maintenance of the corpus luteum.[1-4] The following data lend support to this concept.

Numerous unsuccessful attempts to maintain pregnancy in the hypophysectomized rat with pregnant mare serum (PMS)* led to a trial of other preparations, including lactogenic hormone (LCH),*† a hypophyseal gonadotropic synergist (SY),* and whole anterior pituitary extract (WAP).† Results and relative data appear in Table I. Hypophysectomy was performed between days 5 and 12 of gestation. Pregnancies maintained in rats hypophysectomized prior to the 11th day of gestation may be considered as evidence of successful hormone treatment, since ablation of the hypophysis before the 11th day will terminate pregnancy,[5] owing to atrophy of the corpus luteum.

Of 15 hypophysectomized animals pregnancy was not maintained in 7. Four of the failures were treated with PMS (1 or 2 R.U. daily) and 3 were treated with LCH (2 or 3 mg daily). It should be noted that one of the latter 3 (No. 5) was hypophysectomized only 5 days after mating, and before the expected time of implantation of the fertilized ova.

Pregnancy was maintained in 8 animals from which the pituitary had been removed 7-9 days after mating. LCH (2-3 mg daily) caused maintenance in 6 instances, and SY (3 mg daily) and WAP

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‡ This study was aided in part by Works Project Administration No. 665-51-3-225.

[1] Dresel, I., *Science*, 1935, **82**, 173.

[2] Lahr, E. L., and Riddle, O., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 880.

[3] Evans, H. M., Simpson, M. E., and Turpeinen, K., *Anat. Rec.*, 1938, **70** (suppl.), 26.

[4] Pfeiffer, C. A., *Anat. Rec.*, 1941, **79** (suppl.), 50.

* Pregnant mare serum, hypophyseal synergist, and some lactogenic hormone were supplied by Dr. Erwin Schwenk, Schering Corp., Bloomfield, N.J.

† Most of the lactogenic hormone and the whole anterior pituitary extract used in this work were supplied by Dr. J. B. Schooley, Difco Laboratories, Inc., Detroit, Mich. Thanks are also due Dr. Schooley for his help in obtaining 4 pregnant rats.

[5] Pencharz, R. I., and Long, J. A., *Am. J. Anat.*, 1933, **53**, 117.

TABLE I.
Effect of Chorionic Gonadotropin and Anterior Pituitary Extracts on Maintenance of Pregnancy in Hypophysectomized Rats.

Rat No.	Days pregnant at time of hypophysectomy	Treatment			Litter born (days after mating)	No. of young in litter	Adrenals (g)
		Substance	Daily dose in 1 cc of vehicle	No. of days continued			
1	12	PMS	2 R.U.	14	—	—	—
2	9	"	1 "	13	—	—	—
3	8	"	2 "	15	—	—	—
4	8	"	2 "	8	—	—	.030
5	5	Prolactin No. 35*	3 mg	17	—	—	.022
6	8	" S‡	3 "	15	—	—	.021
7	8	" 35	2 "	16	—	—	.035
8	7	" No. 35771 C†	3 "	17	22	5	.024
9	7	Prolactin S	3 "	17	24	§	.022
10	8	" No. 35	2 "	14	23	8	.029
11	9	" "	2 "	13	22	7	.036
12	9	" "	2 "	13	22	7	.035
13	9	" No. 35771 C	2 "	14	23	7	.044
14	7	Synergist	3 "	17	‖	10 (dead)	.030
15	9	Whole AP	2 "	16	‖	6	.035
16	Normal	—	—	—	22	9	.071
17	"	—	—	—	22	4	.062

*Prolactin (No. 35) was a Difco lactogen assaying 10 I.U. per mg and free of luteinizing hormone.

‡Prolactin (S) was a Schering lactogen.

†Prolactin (No. 35771 C) was a Difco lactogen assaying 20 I.U. per mg and ¼ unit of luteinizing hormone.

§Litter eaten at birth.

‖Not delivered after 26 days.

(2 mg daily) were successful in the one case where each was used. Presumably the corpus luteum—stimulating factor, which Astwood[6] has termed luteotrophin, was present not only in whole anterior pituitary extract, but also in purified lactogenic and gonadotropic synergist fractions.

It will be observed that normal delivery of litters occurred on days 22, 23, or 24 of gestation despite continuation of hormonal administration, except in 2 instances. The rats treated with WAP and SY did not deliver. The former died 26 days after mating and the latter, in a weakened condition, was killed 27 days after mating. Whether any significance can be attached to the fact that these animals did not deliver, while lactogen-treated animals did, will have to be determined by further studies.

When pregnancy was maintained mammary glands revealed slight to marked degrees of stimulation. Lactation was scanty or entirely lacking, and litters died 1 or 2 days after birth. The adrenals of some of the animals treated with LCH were somewhat stimulated as indicated by their weights, while others were atrophic.

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[6] Astwood, E. B., *Endocrinology*, 1941, **28**, 309.

Summary. Pregnancy in rats hypophysectomized on or before the 9th day of gestation was maintained with lactogenic hormone, pituitary gonadotropic synergist, and whole anterior pituitary extract. Each of these substances maintained pregnancy presumably by its ability to stimulate function of the corpus luteum.

13060 P

A Natural Antibody Reacting with Sedimentable Constituents of Normal Tissues.

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While making serological studies of several transplanted rabbit cancers by the methods that disclosed a distinctive substance in the Brown-Pearce carcinoma,[1] we have noted that the blood serum of normal rabbits will fix complement in mixture with saline extracts of normal rabbit tissues in dilutions far beyond any anticomplementary or summative effects. This phenomenon appears to be due to a natural antibody that reacts with sedimentable constituents of normal tissues.

The antibody can be detected by means of a standardized complement fixation test in which 2 units of complement are employed and 2 hours at room temperature allowed for fixation. Antigens are prepared by extracting normal rabbit tissues (liver, kidney, brain, etc.—either fresh or preserved frozen at –22°C) with sand in a mortar, suspending the ground paste in physiological saline (1:10 to 1:40 or more) and centrifuging at 4400 rpm for 20 minutes. The unheated supernatant liquids, opalescent but free from gross particles, have proved notably effective in the tests.

When the sera of 22 normal adult rabbits were mixed with antigens consisting of 1:20 or 1:40 saline extracts of normal rabbit livers, fixation invariably occurred, and it was complete with many of the sera in dilutions as high as 1:16 to 1:64. No fixation was got when the sera of 13 rabbits less than 4 weeks old from 7 litters were tested concurrently with the same antigens. None of the sera proved anticomplementary in dilutions of 1:2 or more in concurrent tests though a few showed slight anticomplementary effect when a double volume of the 1:2 dilution was tested, and the antigens were not anticomplementary when 4 and 8 times the standard amount were employed in control tests.

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[1] Kidd, John G., *J. Exp. Med.*, 1940, **71**, 335, 351.

Experiments were next undertaken to ascertain some of the properties of the substance present in the serum of normal adult rabbits which is responsible for the fixation. It was found that the substance could be precipitated from sera with ammonium sulfate, like the generality of antibodies. The substance was completely inactivated in sera heated at 65°C for 30 minutes—a procedure that has no noteworthy effect upon the generality of antibodies in rabbit blood.[2] But the fact has long been recognized that natural antibodies are more labile to heat than induced ones;[3] hence the substance under study was compared with 2 natural antibodies present in the serum of normal adult rabbits, *i. e.,* natural anti-sheep hemolysin and natural Wassermann reagin. Tests with the sera of 5 rabbits showed that in general the titers of the 3 serum constituents varied together from individual to individual, and that all were inactivated largely or completely by heating at 65°C for 30 minutes, though not apparently affected at 56°C. Sheep erythrocytes absorbed the natural antisheep hemolysin without affecting the titers of either the natural Wassermann reagin or the unknown substance; absorption with cholesterolized Wassermann antigen removed the natural Wassermann reagin from the sera without affecting their content of the natural hemolysin or the unknown substance; and a saline extract of normal rabbit liver absorbed the unknown substance without removing any of the natural hemolysin and but very little of the natural Wassermann reagin.* The findings as a whole would appear to warrant the conclusion that the substance responsible for the fixation of complement in mixture with a saline suspension of normal rabbit liver is a natural antibody.

Saline extracts of various normal rabbit tissues (kidney, liver, brain, lung, testicle, spleen, muscle) will fix complement regularly in dilutions ranging from 1:10 to 1:1280 in mixture with sera known to contain the natural antibody. Kidney and liver antigens have invariably done so in high dilutions whereas heart and voluntary-muscle extracts have reacted only in the lower dilutions. Saline extracts of normal rabbit tissues reacted notably well with serum from the same individual. When extracts of normal rabbit kidney (fresh) and liver (frozen) were spun at 20,000 rpm or more for 60 minutes little or none of the effective antigen remained in the supernatant liquids, but the resuspended sediments were fully as potent as the whole extracts. Alcoholic extracts of rabbit liver and heart muscle failed to react with sera containing the natural antibody, a finding which contrasts significantly with the good fixation obtained when these antigens were mixed with the sera of rabbits having experimental syphilis. The antigen reacting with the natural antibody is adversely affected by glycerol as also upon standing in saline suspension for several days in the refrigerator or upon heating at 56°C for 30 minutes—characteristics which account in part for the fact that its effects were not observed in control tests of the previous studies.[1]

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[2] Jones, F. S., *J. Exp. Med.,* 1927, **46**, 291.

[3] Landsteiner, K., and Reich, M., *Z. Hyg. u. Infektionskrankh.,* 1908, **58**, 213; Mackie, T. J., and Finkelstein, M. H., *J. Hyg.,* 1930, **30**, 1; Gibson, H. J., *J. Hyg.,* 1930, **30**, 337.

* Visible flocculations were not observed in the absorption-mixtures, which were kept 2 hours at 37°C and then put into the refrigerator overnight.

Rabbit sera containing the natural antibody also have the capacity to fix complement in mixture with high dilutions of saline extracts of normal tissues procured from heterologous species (rat, mouse, guinea pig, chicken), and this capacity can be absorbed with normal rabbit-liver extract, though rabbit erythrocytes fail to remove it. Further tests are under way to define more clearly the affinities of the natural antibody and the properties of the substances with which it reacts.

13061 P

Distinct Types of Antibodies in the Blood of Rabbits Carrying the Transplanted V2 Carcinoma.

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From the Laboratories of The Rockefeller Institute for Medical Research, New York City.

Rabbits carrying the transplanted V2 carcinoma—a squamous-cell cancer derived originally from a virus-induced papilloma—regularly develop in their blood an antibody capable of neutralizing *in vitro* the papilloma virus (Shope) and capable also of fixing complement in mixture with it.[1] Further studies have now shown that the virus-neutralizing and complement-fixing titers of sera procured from rabbits carrying the V2 carcinoma invariably parallel one another, and that the antibody responsible for both reactions can be readily absorbed from the sera upon admixture with the virus. From the findings it appears certain that the antibody is directed against the papilloma virus *per se,* and that it is identical with the antiviral antibody present in the blood of rabbits carrying benign virus-induced papillomas, which is in turn identical with that present in the blood of rabbits injected with purified papilloma virus.[2] In further serum tests, carried out by the methods used in the serological study of the Brown-Pearce tumor,[3] we have recently encountered in the blood of rabbits with the transplanted V2 carcinoma two other types of antibodies, wholly distinct from one another and from the antiviral antibody just described.

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[1] Kidd, J. G., and Rous, P., *J. Exp. Med.*, 1940, **71**, 813.

When the sera of normal adult rabbits and of rabbits carrying the V2 carcinoma are heated at 56°C for 30 minutes and tested for capacity to fix complement in mixture with unheated 1:20 or 1:40 saline extracts of normal and neoplastic tissues (*e. g.,* normal rabbit liver or kidney, V2 carcinoma, Brown-Pearce carcinoma) complement-fixation takes place in nearly all of the mixtures.* In general the normal sera react in dilutions as high as 1:16 to 1:32 with the normal tissue extracts, and only about half as well with the neoplastic-tissue antigens. On the other hand, sera from rabbits that have carried progressively enlarging V2 carcinomas for some weeks not only react with normal tissue extracts but are vastly more potent in mixture with the homologous antigen, generally fixing 2 units of complement completely in dilutions as high as 1:128 or 1:256 or more in mixture with extracts of the V2 carcinoma. (The sera show no anticomplementary effects in dilutions of 1:2, nor do the antigens when tested simultaneously in double volume.) When heated at 65°C for 30 minutes, the sera of normal rabbits lose completely their capacity to react with either the normal or the neoplastic tissue antigens. The sera of rabbits carrying the V2 carcinoma, by contrast, retain undiminished their capacity to react with the V2 carcinoma antigen, though their ability to react with the normal tissue and heterologous tumor antigens is abolished or markedly diminished.

The heat-resistant antibody capable of fixing complement specifically in mixture with saline extracts of the V2 carcinoma was regularly encountered in the blood of rabbits that had carried progressively enlarging growths for periods of several weeks (18 rabbits). It was not present in the blood of normal animals (22 rabbits), in that of rabbits recovering from vaccinia, virus-induced fibromatosis, or experimental syphilis (15 rabbits), nor in that of rabbits carrying transplanted cancers of other types. It can be readily differentiated from the natural antibody, which is also present in the blood of normal adult rabbits, both by its specific affinity for the V2 carcinoma antigen and by the fact that it is unaffected by heating at 65°C for 30 minutes, a procedure that inactivates the natural antibody.[4]

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[2] Friedewald, W. F., and Kidd, J. G., *J. Exp. Med.,* 1940, **72**, 531.

[3] Kidd, J. G., *J. Exp. Med.,* 1940, **71**, 335, 351.

* For a brief outline of the complement-fixation procedure, see the accompanying paper.[4]

The antibody that reacts with extracts of the V2 carcinoma is not the same as that reacting with the papilloma virus, though the 2 occur together in the same sera, often in high titer. Both are heat-resistant, but they can be readily discriminated by their differing affinities. The V2 antibody reacts with extracts of the V2 carcinoma but not with purified suspensions of the papilloma virus, while the antiviral antibody reacts with the virus (and can be selectively absorbed thereby) as not with extracts of the V2 carcinoma.

The experiments have disclosed 3 distinct types of antibodies in the blood of rabbits carrying the V2 carcinoma. Similar studies have been undertaken with other transplanted cancers.

13062

Excretion of Sulfanilylguanidine in Material Drained from Human Biliary Tract.

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Recently a new sulfonamide drug, sulfanilylguanidine, has been developed and studied by Marshall and various other workers.[1] Although the solubility of this drug is not markedly different from that of various related compounds, it appears to be absorbed rather slowly from the gastro-intestinal tract. Because in recent studies of the excretion of various dyes by the liver it has been found that marked differences in excretion occur which cannot at present be

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[4] Kidd, J. G., and Friedewald, W. F., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 128.

[1] Marshall, E. K., Jr., Bratton, A. C., White, H. J., and Litchfield, J. T., Jr., *Johns Hopkins Med. Bull.*, 1940, **67**, 163.

TABLE I.
Sulfanilylguanidine in Blood Bile and Urine.

	Time	Blood, mg/100 cc	Bile, mg/100 cc	Urine, mg
Case I	6 A.M.	—	.0*	—
	8 ”	1.2	—	—
	10 ”	—	.5	2.6
	12 M.	1.8	—	—
	2 P.M.	—	.7	17.7
	4 ”	1.0	—	—
Case II	10 A.M.	0.3	.0*	—
	2 P.M.	0.5	.2	—
	4 ”	—	.1	—
	6 ”	0.2	—	52.0†

2 g sulfanilylguanidine ingested at 6 A.M. by each patient.
*Less than 0.1 mg/100 cc.
†12-hour sample of urine.

correlated in a satisfactory manner with either solubility or diffusibility of the dyes,[2] it seemed possible that the apparent failure of absorption of sulfanilylguanidine might be really due to a removal of the drug from the blood stream by the liver, and a return of the material to the gastro-intestinal tract in the bile flowing through the biliary ducts. At about the time when a supply of sulfanilylguanidine became available for experimental purposes 2 patients were operated on for lesions of the biliary tract, and T tubes inserted into the ducts to provide post-operative drainage. It was decided to study the excretion of sulfanilylguanidine through the bile ducts of these patients. Two grams of sulfanilylguanidine were therefore given to each, and its concentration in the blood, urine and bile determined. The methods previously described[3] were found to be quantitatively applicable to the study of sulfanilylguanidine and were used in these experiments. The results are given in Table I. One of the patients showed a moderate, and the other a minimal concentration of the drug in the blood. It is evident that in neither instance was there any evidence that unusually great concentrations of sulfanilylguanidine occurred in the bile. The ratio between the concentrations in blood and bile were essentially similar to those found in the previous study of sulfapyridine. The thesis that the apparent failure of absorption of sulfanilylguanidine resulted in part from a marked reëxcretion of the drug into the intestine therefore proved incorrect.

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[2] Haber, R., *J. Gen. Physiol.*, 1940, **23**, 185.

[3] Hubbard, R. S., and Butsch, W. L., PROC. SOC. EXP. BIOL. AND MED., 1940, **46**, 484.

13063 P

Phosphorus Exchange in Tissues of Patients with Lymphoid Leukemia.*

L. A. Erf† and G. Friedlander. (Introduced by J. H. Lawrence.)

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Methods and Materials. Radio-phosphorus (P^{32}) produced by the Berkeley cyclotron[1] was converted into sodium phosphate for oral and intravenous administration as therapy to patients with leukemia.[2]

Small pieces (about 5 g) of tissues obtained at the autopsy of 4 patients so treated were placed in crucibles and ashed at 400°C. The radioactivity of the ashes was measured by means of an ionization chamber. All measurements were calibrated by means of a uranium standard and the activities were corrected for rate of decay of radio-phosphorus (half-life, 14.3 days) to date of death. The total phosphorus (P^{31}) content of the ashes was determined by the method of Pregl.[3] By dividing the amount of radioactivity (microcuries) per gram wet weight of each tissue by the number of milligrams of phosphorus (P^{31}) per gram wet weight of the same tissue, one obtains the "specific radioactivity" of the tissue in μc/mg P^{31}.

Results. The results are listed in Table I. The notable but anticipated feature is that the "specific radioactivity" of the listed tissues was independent of the variations in type of disease, age of patient, quantity‡ or route of administration of radio-phosphorus, quantity of ingested nonradioactive phosphorus (including that in the diet) and quantity of P^{31} and P^{32} per gram of a specific tissue, but was apparently dependent upon the time interval between the last dose of radio-phosphorus and death. The quantity of P^{31} in some of the tissues of normal individuals[4] are also recorded in the table.

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* This investigation was aided by the Blanche and Frank Wolf Foundation.

† Wm. R. Kenan, Jr., Fellow.

[1] Lawrence, E. O., and Cooksey, D., *Phys. Rev.*, 1936, **50**, 1131.

[2] Lawrence, J. H., Scott, K. G., and Tuttle, L. W., *New International Clinics*, 1939, **3**, 33.

[3] Pregl, F., and Roth, H., *Quantitative Organische Analyse*, Julius Springer, Berlin, 1935.

‡ The doses of radio-phosphorus administered were "therapeutic" and not "tracer" in quantity.

[4] Schmidt, C. L. A., and Greenberg, D. M., *Physiol. Rev.*, 1935, **15**, 382.

TABLE I.

Case No.	1.			2.			3.			4.			Normal Individuals*	
Age (yr)	4			11			67			24				
Type of lymphoid leukemia	acute leucopenic			acute leucopenic			chronic leucocythemic			chronic leucocythemic				
Avg white blood cell (per mm^3)	3000			5000			100,000			60,000				
Dates, routes of adm.† and amts (Mc)‡ of P^{32} administered	2/13—1 Mc—I.V. 2/17—1 Mc—I.V.			1/4 to 3/9 14.09 Mc—I.V. 1.73 Mc—O.			10/20—20 Mc—O			12/14 to 2/24 44.1 Mc—O				
Total sodium phos. in which P^{32} was incorporated (g)	1.65			1.35			3.60			20.61				
Days bet. last adm. of P^{32} and death	11			18			19			2				
Tissues studied and results (μc)§	μc/g wet wt	mg P^{31}/g wet wt	μc/mg P31	μc/g wet wt	mg P^{31}/g wet wt	μc/mg P31	μc/g wet wt	mg P^{31}/g wet wt	μc/mg P31	μc/g wet wt	mg P^{31}/g wet wt	μc/mg P31	mg P^{31}/g wet wt Min.	mg P^{31}/g wet wt Max.
1. Cerebellum				.058	3.37	.0174	.030	2.72	.0092	.053	2.78	.019	1.40	1.80
2. Kidney	.069	1.63	.043	.151	3.74	.0404	.065	1.21	.0446	.229	1.78	.127	1.27	1.92
3. Liver	.074	1.89	.039	.128	3.11	.0412	.088	1.57	.0467	.368	2.09	.176	1.84	3.05
4. Lung	.054	1.86	.029	.039	1.27	.0308	.042	1.20	.0297	.176	1.47	.119		
5. Lymph nodes	.058	1.78	.033	.154	3.96	.0389	.076	1.63	.0389	.320	2.67	.119		
6. Muscle														
a. Heart	.058	1.69	.034				.040	1.16	.0291	.155	1.41	.109	1.42	2.99
b. Skeletal	.039	1.40	.028				.046	1.77	.0221	.142	1.58	.090	1.00	2.00
7. Spleen	.075	.65	.116	.088	2.52	.0350	.066	1.60	.0348	.276	2.02	.136		
8. Sternum	.088	17.28	.005	.081	32.5	.0025	.078	19.9	.0032	.277	35.0	.0078		

*Bibliography, No. 4. †O = oral; I.V. = intravenous. ‡Mc = millicuries. §μc = microcuries.

Discussion. When one contrasts the "specific radioactivities" of Case 4 with those of the other 3 cases it becomes apparent that fairly large amounts of P^{32} have been retained by the various tissues at the end of the second day after administration. Apparently, however, a large part of the quantity leaves the tissues between the second and eleventh day. That retained is apparently specific for each type of tissue since the "specific radioactivities" of each tissue of the 3 cases were similar and were independent of the total amount (within the limits listed) of P^{32} administered and the other factors mentioned above. The quantities of phosphorus appearing in the tissue during the first few days probably take part in "carbohydrate" metabolism while those retained for longer periods of time may be involved in "nucleoprotein" and "phospholipid" metabolism.[5]

13064 P

Photoelectric Microdetermination of Calcium in Serum.

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A new microcolorimetric method has been developed for the determination of calcium by direct precipitation, in samples of 0.2 cc or less of serum. Although work is now in progress on a simplification of the technic and the use of smaller samples for analysis, the following procedure has given, for some time, such satisfactory results (accuracy within ±2%) as to warrant its present description.

Procedure. In test tubes (10 x 75 mm), to 0.2 cc samples of serum are added 1.0 cc of water and 0.2 cc of saturated ammonium oxalate. Two *blank* samples, with water in place of serum, are treated in exactly the same way. After standing overnight, 0.2 cc of 0.1% Triton N E (Röhm and Haas) is added to each tube,[1] the contents stirred, then poured into a pyrex sintered glass filter funnel (No. 2 F, Buchner type) held by a rubber stopper in the mouth of a 500 cc suction flask. Three successive portions of 0.8 cc of 2% ammonia water are pipetted into the tube, agitated to pick up any remaining crystals of CaC_2O_4, then poured upon the filter. A receiving tube (7 x ⅞") is placed in the suction flask under the stem of the filter funnel. The vacuum is turned off, while the walls of the precipitation tube are washed with 1 cc of hot 1 *N* H_2SO_4 which is then poured on the filter and allowed to stand for one minute. Suction is reapplied and the acid is caught in the receiving tube. The precipitation tube is discarded. The walls of the filter funnel are then washed with 1 cc and 2 cc portions of hot 1 *N* H_2SO_4, allowed to stand for one minute as above.

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[5] Tuttle, L. W., Erf, L. A., and Lawrence, J. H., *J. Clin. Invest.*, 1941, **20**, 57.

[1] Alter, C. M., and Thomas, D. S., Jr., *Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 525.

The 4 cc of acid in the receiving tube are transferred to a 50 cc volumetric flask, with 3 washings of 3 cc of water. To the 2 blanks, *1* and *2*, which have been washed and filtered in exactly the same way as the serum samples, in the 50 cc flasks there are added 1 cc and 2 cc, respectively, of freshly prepared 0.000625 *N* $Na_2C_2O_4$ (1 cc of 0.125 *N* diluted to 200 cc). To each flask there is then added 1 cc of 0.0018 *N* $Ce(SO_4)_2$ (9 cc 0.1 *N* diluted to 500 cc in 1 *N* H_2SO_4). After mixing, the flasks are allowed to stand for 30 minutes at room temperature. One cc of freshly prepared 0.5% KI is added to liberate free iodine,[2] allowed to react for 60 seconds, then water is added to approximately 40 cc volume. The contents are brought to temperature of 25°C, and 0.5 cc of 2% starch in saturated NaCl solution is slowly added, with gentle mixing by rotation. Water is added to volume, and 15 cc portions of the blue solution are read at 25°C in the Evelyn colorimeter,[3] with a No. 600 filter in place.[4] The center setting is obtained with a pure water tube set at 100.

The readings are plotted on semi-logarithmic paper (K. and E. No. 358-51) with abscissa units of micro-equivalents oxalate per liter from 0 to 35, and ordinate units of galvanometer readings from 10 to 100. A straight line is drawn between the readings for the two *blanks 1* and *2*, representing 12.5 and 25 micro-equivalents per liter, respectively, of oxalate. The oxalate values for the serum samples found by interpolation of the galvanometer readings on this line, when multiplied by 0.25 or 0.5 give directly Ca as milli-equivalents per liter, or mg per 100 cc, respectively, in the original serum samples.

Results. Samples of human serum analyzed as above *(C)* and by gasometric analysis *(G)* of 1 cc samples directly precipitated,[5] in terms of mE. Ca per liter serum, gave the following typical results:

Sample	1	2	3	4	5	6	7	8	9	10
G	4.83	4.97	4.51	4.90	5.06	4.84	5.28	4.92	5.12	5.12
C	4.80	5.00	4.35	4.80	5.20	4.81	5.23	4.86	5.07	5.17

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[2] Groák, B., *Biochem. Z.*, 1929, **212**, 47.

[3] Evelyn, K. A., *J. Biol. Chem.*, 1936, **115**, 63.

[4] Sendroy, J., Jr., *J. Biol. Chem.*, 1939, **130**, 605.

[5] Van Slyke, D. D., and Sendroy, J., Jr., *J. Biol. Chem.*, 1929, **84**, 217.

The average deviation of the micro (0.2 cc) colorimetric results from the macro (1 cc) gasometric values was ±1.5%, with extreme limits of –3.6 and +2.8%.

13065 P

Electro-Encephalographic Diagnosis of Extradural and Subdural Hemorrhage.

MARK ALBERT GLASER AND HENDRICKUS SJAARDEMA. (Introduced by C. H. Thienes.)

From the Research Laboratory, Cedars of Lebanon Hospital, Los Angeles, California.

It has been our belief that abnormal waves produced by pressure lesions upon the surface of the brain, such as extradural or subdural hemorrhage, etc., would differ from those wherein the brain was actually damaged or destroyed by laceration, contusion or tumor invasion. In order to determine these facts, we have studied, to date, 30 experimental animals, retesting them for 4 months, and 3 clinical cases of verified acute and subacute hematomas.

In 3 patients with verified subdural hematoma, 2 were subacute and one was acute; the electro-encephalographic diagnosis demonstrated delta waves of 1 cycle per second, with a voltage of 100 microvolts, combined with a rapid wave of 16 cycles per second, and a voltage of 20 microvolts. This pattern was localized over the hemorrhage. Following the surgical removal of these hemorrhages, the waves immediately returned to normal in the acute case. Six months later the 2 subacute cases showed normal waves. In the acute case the test was repeated within 12 hours after the removal of the hemorrhage. The high beta waves had disappeared and the 1 and 2 cycle delta waves of 100 microvolts had changed to 2 to 4 cycle deltas of 75 to 150 microvolts. This type of wave was found in all leads.

The normal electro-encephalogram of the rabbit, as obtained through the intact skull, was established by 150 recordings. No. 5 gelatine capsules, covered with animal membrane, were filled with citrated rabbit's blood. In one-half of these capsules thorotrast was added to make the capsule opaque to the roentgen ray. This addition of thorotrast did not affect the electrical waves. Under aseptic pro-

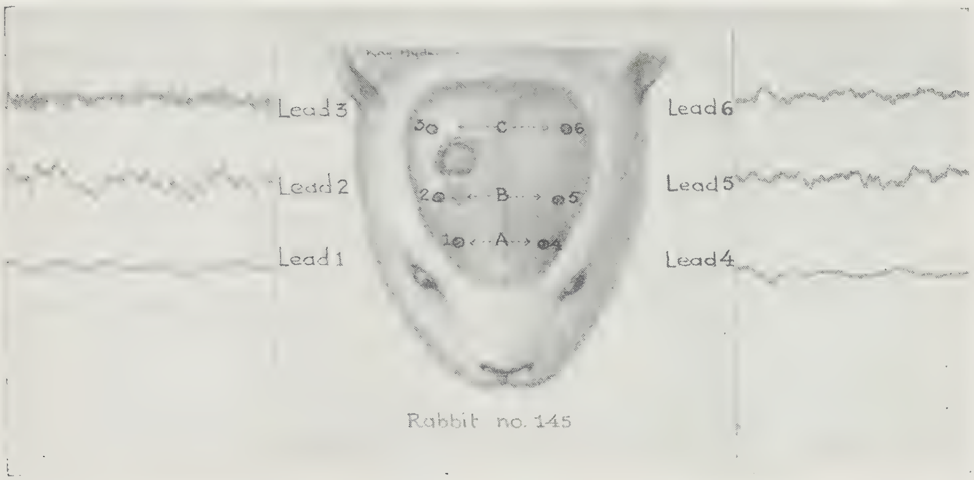

FIG. 1.
Characteristic waves found immediately after insertion of capsule to four months post-surgical.

caine anesthesia the capsules were inserted subdurally in some animals and extradurally in others.

The following wave patterns (Fig. 1) appeared immediately after insertion of the capsule and have persisted for a period of 5 months: (1) The 4 to 6 cycle per second waves of 60 microvolts were absent in all animals; (2) the 16 and 30 cycle per second waves of 5 microvolts were increased to 20 microvolts; (3) a combination pattern consisting of 2 to 4 cycle waves of 50 to 150 microvolts wherein 16 to 30 cycle waves were superimposed upon the 2 and 4 cycle waves.

The electro-encephalogram recorded immediately after removal of a capsule one week after insertion was entirely normal, except that the 4 to 6 cycle per second waves did not appear in those animals wherein they were originally present.

The electro-encephalogram taken immediately after removal of the capsule, which had been inserted 2 weeks prior, showed a gradual decrease in the abnormalities. Within one week's time the waves were entirely normal. The abnormalities found were as follows: (1) The 4 to 6 cycle per second waves of 60 microvolts were absent in all animals; (2) the 16 and 30 cycle per second waves of 5 microvolts were increased and ranged around 10 microvolts; (3) the 2 to 4 cycle per second waves were reduced and ranged around 75 microvolts. The same combination wave existed. In the rabbits, wherein the capsules were removed from 3 to 4 weeks after insertion, the same decrease in voltage occurred as in the 2-weeks group.

At a later date, we will report the length of time necessary for these capsules to remain in the brain to cause permanent damage, the type of abnormal waves associated with cortical and subcortical trauma, and the existence of these patterns in meningioma, gumma, tuberculoma and hydrocephalus.

Conclusions. Three clinical cases of subdural hematoma, and 30 rabbits wherein capsules had been inserted, both extradurally and subdurally, demonstrated a consistent wave pattern.

13066 P

A Comparative Study of Rodent and Burrow Flea Populations.*

M. A. STEWART AND F. C. EVANS. (Introduced by K. F. Meyer.)

From the Division of Entomology and Parasitology, University of California, Davis, and the George Williams Hooper Foundation for Medical Research, University of California, San Francisco.

An area located at Calaveras Dam, Alameda County, California, previously determined to be heavily populated with ground squirrels [*Citellus beecheyi* (Richardson)], was selected for field study of (1) the correlation between flea populations existing at squirrel burrow openings and of flea populations on the rodent hosts, and of (2) the seasonal species composition of these populations. One portion of this area was set aside for the study of the fleas at the openings of the burrows, and a contiguous and ecologically similar portion was utilized for the study of fleas taken directly from their hosts.

Fleas at the burrow openings were collected from pieces of cotton batting placed in each burrow mouth. Thirty burrows were set aside for these studies, divided into 3 groups of 10 burrows each and approximately equally distributed as to direction of exposure, amount of exposure, surrounding cover crop and topography. Collections were made at approximately weekly intervals from April 29, 1940, to October 23, 1940, as shown in Graph 1, and they were made in regular rotation among the 3 sets of burrows in order not to influence unduly the population densities by repeated collections. In the same period and also at approximately weekly intervals, as shown in Graph 2, ground squirrels were trapped alive, lightly anesthetized with chloroform, and thoroughly combed to remove all fleas. At each burrow collection temperatures were taken at points 1 foot within the mouth of the burrow and 6 feet above the ground. All fleas collected were mounted and identified; of approximately 7500 fleas collected only about a dozen belonged to species other than *Diamanus montanus* (Baker) or *Hoplopsyllus anomalous* (Baker).

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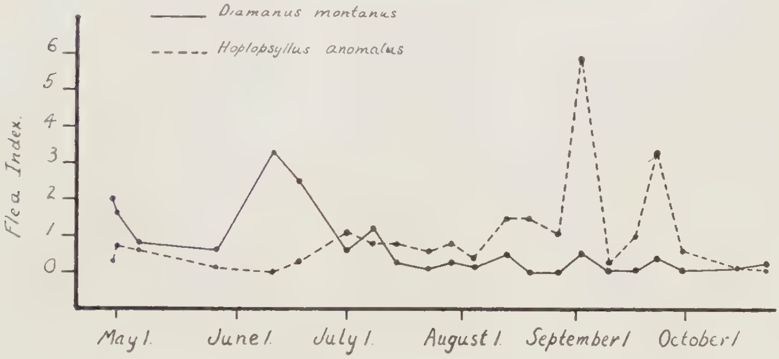
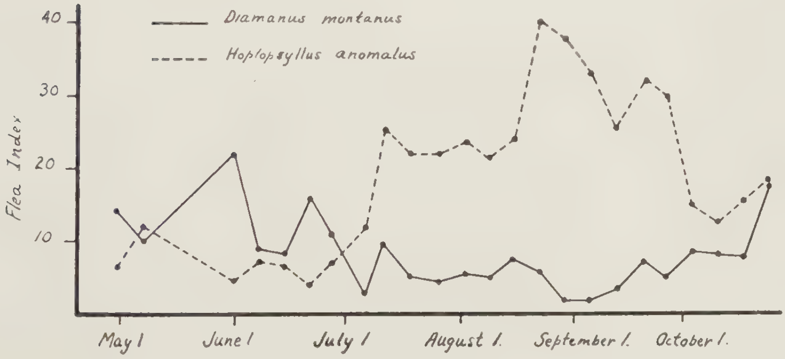
* This study was conducted with the financial assistance of the Rosenberg Foundation. The authors wish to express their appreciation to Dr. K. F. Meyer, Director of the George Williams Hooper Foundation, for his constant advice and suggestions, to Dr. E. B. Roessler, Head of the Division of Mathematics and Physics, University of California, Davis, for valuable assistance in the analysis of data, and to Robert Holdenried for assistance in collecting material in the field.

As shown in Graphs 1 and 2, *Diamanus montanus* was markedly predominant in the flea population from April 29 to June 28 and *Hoplopsyllus anomalus* was markedly predominant from July 11 to



Graph I. Flea collections from mouths of burrows.



Graph II Flea collections from ground squirrels.

October 14. No correlation could be detected between either atmospheric or burrow temperatures and flea indices other than that *Diamanus montanus* was predominant while the mean temperature was below 75°F and *Hoplopsyllus anomalus* was predominant while the mean temperature was above 75°F.

Comparison of Graphs 1 and 2 shows that the flea population density and species composition at the mouths of burrows reflects with a high degree of accuracy, but on a smaller magnitude, the population density and species composition on the host. Since each collection shown in Graph 1 represents the index of 10 burrow openings, it apparently can be safely assumed that flea collections made from a series of 10 or more mouths of inhabited burrows will portray the flea population density and its species composition on rodents occupying such burrows in a given area. It is, therefore, quite feasible to make flea surveys for the purpose of obtaining information concerning the appropriate time to initiate prophylactic practices against sylvatic plague, the chronological predictions of epizootics, the plague potentialities of an area based upon the species composition of the flea population, and, presumably, the existence or absence of plague infections in the flea population by collecting specimens from burrow openings.

A distinct difference in seasonal incidence exists, at least at Calaveras Dam and probably in all other places as well, between *Diamanus montanus* and *Hoplopsyllus anomalus*. Although *H. anomalus* has been observed to transmit plague under experimental conditions, it seems likely that this species is not as efficient a vector of plague as is *D. montanus*. Because of this and the difference in seasonal distribution of flea species, plague surveys based upon flea collection should be correlated with those times of year when species capable of transmitting the infection are abundant. It is quite possible that many of those areas heretofore recorded as being free from plague infection have been placed in this category because they were surveyed at a time when efficient vectors had been largely replaced by species which are either very poor vectors or incapable of transmitting plague.

13067

Changes in Chemical Composition of the Blood Coming from Damaged Tissues.*

HANS SELYE AND CRISTIANE DOSNE.

From the Department of Anatomy, McGill University, Montreal, Canada.

Among the various theories put forward to explain the symptoms of "shock",[1] the toxic theory appears to have the largest number of supporters at the present time. It assumes that a toxic metabolite or metabolites (histamine or other protein decomposition products) are liberated from the damaged area into the blood and secondarily produce the systemic changes typical of this condition. However, it proved impossible to demonstrate the presence in the blood of toxic amounts of such metabolites during shock. Perhaps the best evidence in favor of the toxic theory is that in the case of temporary occlusion of all the blood vessels leading to an extremity, no condition of shock develops as long as the vessels remain occluded—even though the extremity is severely damaged as a result of anoxia—but release of the circulation causes rapid development of shock. This is supposedly due to intoxication of the body with toxic metabolites discharged from the damaged limb.

In a series of experiments on 6 male Macaca mulatta monkeys weighing 7 to 9 lbs, we confirmed these observations. We noted that if a rubber tourniquet is placed tightly around the upper part of one thigh for a period of 6 hours no noteworthy systemic damage is produced. The blood pressure and body temperature remained practically normal but about 15 minutes after the release of the ligature, the pulse became very feeble and the blood pressure decreased to such an extent that in most cases it was impossible to measure it. In these animals, the systemic signs of damage occurred before any significant amount of edema was demonstrable in the damaged area. This is important since according to some investigators, loss of fluid through the damaged capillaries in injured parts is the cause of systemic shock. It is remarkable that although no water was given to these monkeys, more severe edema developed in the damaged leg of each of the 3 animals which recovered than in the 3 which succumbed to shock. It is also noteworthy that the

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* The expenses of this work have been defrayed through a grant received from the National Research Council of Canada.

[1] Moon, Virgil H., *Oxford Medical Publications*, 1938.

edema was more pronounced after recovery from shock than at the peak of the shock condition. All these observations speak strongly against the theory that shock is due to loss of fluid into the damaged area but are apparently explicable on the basis of the toxic theory.

Since there is no definite direct proof in favor of the toxic theory, we performed some experiments in which the blood coming from the damaged area was directly injected into adrenalectomized mice which are particularly sensitive test objects for the detection of toxic substances. Rabbits were used as donors. In a series of 3 rabbits, the femur was disarticulated at its cranial end and all soft tissues excepting the femoral artery and vein were ligated with a thread placed around them under the skin in the region of this articulation. The femoral vein was then ligated with a separate ligature and the artery temporarily occluded by placing a protective cuff of rubber around it and ligating on the rubber. This operation was performed under ether anesthesia from which the animals were allowed to recover after the intervention. Five hours later the ligature occluding the artery was released under a second ether anesthesia while blood was taken from the femoral vein of the damaged as well as the normal leg at the same time. In order to avoid blood clotting, a few drops of heparin were injected into the artery of the damaged leg at the beginning of the experiment simultaneously with the ligature of the vein. Just before bleeding the animal, 0.5 cc of heparin was injected into the general circulation. The blood collected from the 2 legs was then comparatively assayed by injecting 0.5 cc into each of 15 adrenalectomized mice weighing 25 to 30 g. The death rate among the animals receiving blood coming from the normal extremity was approximately the same as among those injected with the blood coming from the damaged leg. Thus no special toxicity could be demonstrated even in the blood derived directly from a leg which was so severely damaged by anoxia that had its circulation been reunited with the rest of the body, severe shock would have developed.

Although these experiments are not explicable on the basis of the toxic or the dehydration theory of shock, they are not incompatible with the theory proposed by one of us[2] according to which shock may be caused by "the lack of something rather than the excess of a toxic metabolite." It is indeed quite possible that damaged tissue actively attracts certain blood components of vital importance and thus depletes the rest of the body of an important substance or substances, the deficiency of which causes shock. It will be remembered that our understanding of vitamin deficiencies made no progress as long as these were regarded as food intoxications while the recognition of their nature as deficiency diseases—though viewed with great skepticism at the beginning—was the key to most of what we know about vitamins today.

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[2] Selye, Hans, *Cyclopedia of Med.*, 1940, **15**, 15.

In order to subject the deficiency hypothesis of shock to experimental investigation, we deemed it necessary, first, to establish whether damaged tissues are really merely in a catabolic state of metabolism or whether they can actively anabolize certain substances or at least remove them from the blood, more actively than normal tissues. For this purpose we made a preliminary experiment on 4 male rats weighing 240 to 338 g in which the blood vessels of one hind leg were temporarily occluded for a period of 5 hours using the same method as described above in connection with our experiments on the rabbit. It was found that the blood sugar coming from the vein of the intact leg varied between 100-124 mg % (Hartmann, Schafer, Somogyi method) while the venous blood coming from the leg whose circulation had been occluded contained only 50-72 mg % glucose. We concluded that in certain respects at least, the metabolism of the damaged leg is more active than that of the intact extremity. It appeared possible, however, that most of the blood recovered was that which stagnated in the occluded vessels and that the blood sugar was lowered mainly because the tissues had used all the sugar originally present in this particular portion of blood. It seemed important, therefore, to see whether a damaged leg would continue to remove sugar from the circulation even if the blood flow were reëstablished for a longer time. To test this, we decided to take 2 specimens of blood, one immediately after reëstablishment of the arterial inflow and one about 15 seconds later. Since the blood escaping through the vein during this interval was discarded, it is evident that the second specimen could not have contained blood which had stagnated in the leg during the occlusion period. It also appeared of interest to determine the blood chlorides, hemoglobin, blood N.P.N. and muscle chlorides comparatively in the 2 legs so as to gain more information concerning the biochemical changes which occur in the injured area. At the same time, we wished to determine whether there is any significant difference between the biochemical changes seen after shorter and longer occlusion of the blood vessels. In order to study all these changes, we decided to use the cat which appeared more suitable than the rat for experiments necessitating larger quantities of blood. Table I summarizes our observations.

TABLE I.
Changes in the Chemical Composition of the Blood Coming from Damaged Tissues.

Experimental animal		Pregnant female cat—13 lbs			Female cat—15 lbs			Male cat—6 lbs			Female cat—10 lbs		
Blood derived from	hr	Damaged leg		Intact leg	Damaged leg		Intact leg	Damaged leg		Intact leg	Damaged leg		Intact leg
Hemoglobin	3	17.7	11.2	8.0	11.3	9.8	7.0	19.5	19.5	13.9	13	13.7	9.6
g/100 cc	6	*	*	*	9.8	8.4	7.1	15.8	16.6	11.6	15.8	14.4	7.6
Blood sugar	3	111	154	182	75	124	224	145	107	215	174	107	224
mg/100 cc	6	115	107	182	124	104	182	159	104	182	104	75	166
Blood N.P.N.†	3	75	84	58	81	71	65	75	62	58	56	62	54
mg/100 cc	6	*	*	*	72	77	56	66	64	56	64	58	56
Muscle chlorides mg/100 g	6	234	*	70	196	*	66	176	*	59	135	*	70

*No determination made at this time.

†It will be noted that the N.P.N. values obtained with this micro method are somewhat higher than those obtained with the macro methods. This is merely due to the different technic of preparing the protein filtrate and does not interfere with comparative determinations.

Blood samples were taken for study 3 hours after temporary occlusion of the circulation, then the circulation was occluded again and a second specimen taken 6 hours after the beginning of the experiment. In almost every case, 2 samples were taken from the damaged leg, the first immediately after reëstablishing the circulation and the second about 15 seconds later, after all the blood stagnating in the extremity had been removed. It will be seen that there is no very important difference between the chemical composition of the first and second sample nor between the determinations performed after 3 and 6 hours respectively. There is, however, a very consistent difference in the blood sugar (Hartmann, Schafer, Somogyi method) which is high in the intact leg and normal or subnormal in the damaged leg. The N.P.N. (Folin and Wu method modified for microdetermination with the photoelectric colorimeter) proved consistently higher in the damaged leg than on the other side and similarly hemoglobin (photoelectric colorimeter method) determinations indicated hemoconcentration during the passage of blood through the damaged extremity.

It is evident from these experiments that damaged tissue may utilize certain substances more actively than the corresponding normal tissues. We have no evidence that deficiency in any of the metabolites studied in this experimental series plays an essential rôle in the production of the shock syndrome. Indeed the blood chlorides rarely reach a dangerously low level in fatal shock and the systemic blood sugar is usually above normal rather than below normal in this condition. However, there is no convincing evidence in favor of, but a good deal of observational material incompatible with, the other theories of shock while we are not aware of any observation incompatible with the deficiency theory. Our experiments indicate that increased utilization of certain metabolites (*e. g.*, sugar) can occur in the damaged area. This raises the question whether the systemic symptoms of shock might not perhaps find their explanation in that the active utilization of sugar by the damaged tissue—although unable to deplete the actual sugar reserves of the body—depletes the organism of compounds (enzyme systems, hormones, vitamins?) essential for the utilization of sugar by tissues outside of the injured area. This may perhaps also explain why the systemic blood sugar is high at a time when the blood sugar in the damaged area is low.

Summary. Experiments on monkeys show that in this primate, temporary occlusion of the blood supply to the legs does not cause any signs of shock until the circulation through the damaged leg is reëstablished. Then severe shock ensues even before any significant amount of fluid can be lost from the blood into the damaged area. This observation is incompatible with the dehydration theory of shock.

Blood taken from the vein of an extremity which was damaged by temporary occlusion of its blood supply does not prove more toxic when assayed on the adrenalectomized mouse than blood coming from an intact extremity. This observation is incompatible with the toxic theory of shock.

Experiments on the rat and cat indicate that the sugar concentration of the blood coming from a damaged extremity is significantly below the corresponding values in normal venous blood, conversely the hemoglobin and N.P.N. concentration is above normal in blood which passed through the damaged area. It appears that damaged tissues remove certain metabolites from the blood while they discharge other substances into it. These observations are considered in the light of the hypothesis according to which the general systemic signs of shock are caused by deficiency in certain substances vital for the economy of the body. According to this assumption, shock would follow after the release of temporarily occluded blood vessels not because substances coming from the limb poison the rest of the body but because active metabolic processes drain the body of certain essential compounds, the lack of which causes shock. The authors wish to point out that meanwhile they regard this interpretation merely as a working hypothesis but as such, they feel that unlike the other shock theories, it has the advantage of being compatible with all experimental observations which have so far been made on the subject of shock.

13068

Muscular Dystrophy in the Absence of Testicular Degeneration in Vitamin E Deficiency.*

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As a result of the symptoms leading to its discovery, vitamin E has been defined as a fat-soluble essential preventing a characteristic and specific sterility in the male and female rat. Evans and Burr[1] described paralysis in the nursing young of female rats which had received minimal amounts of vitamin E. Olcott,[2] and later others, observed lesions of the skeletal muscles in the deficient young. However, the appearance of young rats weaned from stock females to a vitamin E-deficient diet is normal except for a relatively slight (compared to most deficiency states) impairment in the rate of growth until they develop paralysis of the rear limbs after many months on the diet. These paralyzed adult animals, according to Einarson and Ringsted,[3] exhibit first microscopic lesions in the central nervous system and later lesions in the voluntary muscles. Recently Knowlton, *et al.*,[4] have reported that in the long period elapsing between the appearance of gross neuro-muscular symptoms in suckling and adult E-deficient rats, chemical and functional changes and occasional necrotic fibers can be detected in the skeletal muscles in the absence of overt symptoms. Nevertheless, for many months of the rat's life, sterility remains the most prominent symptom produced thus far by a dietary deficiency of vitamin E.

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* Supported by a grant from the Research Corporation of New York.

[1] Evans, H. M., and Burr, G. O., *J. Biol. Chem.*, 1928, **76**, 273.

[2] Olcott, H. S., *J. Nutrition*, 1938, **15**, 221.

[3] Einarson, L., and Ringsted, A., 1938, *Effect of Chronic Vitamin E Deficiency on the Nervous System and the Skeletal Musculature in Adult Rats*, Oxford University Press.

[4] Knowlton, G. C., Hines, H. M., and Brinkhous, K. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 453; 1939, **42**, 804.

In contrast to the rat, young rabbits placed on a vitamin E-deficient diet pass through the following sequence in 3 to 6 weeks: abnormal creatinuria, decreased rate of growth, loss of weight, muscular weakness, inanition, and death.[5] Hyalinization and necrosis of the skeletal muscle fibers is extreme. If a partial deficiency is produced by administering subminimal doses of alpha-tocopherol the animals grow for months, eat well and show no gross symptoms.[6] However, during this time the urinary creatine is elevated and on histological examination extensive microscopic lesions are found in the skeletal muscles.

In view of the specific and irreversible nature of the testicular degeneration found in vitamin E-deficient rats,[7] experiments were undertaken to determine whether the testes in adult rabbits suffering from a complete or partial deprivation of E are more or less susceptible to damage than the skeletal muscles.

Seven adult bucks were unilaterally castrated under ether anesthesia. The testes were weighed, fixed and sectioned. All were normal. Five days after the operation the animals were placed on vitamin E-deficient diet No. 13.[5] Three of the rabbits, serving as controls, received 5 mg of alpha-tocopherol[†] in ethyl laurate orally 6 days a week. After a week on the diet one of the controls, No. 120, persistently refused food.

All of the deficient animals eventually lost weight and developed symptoms of dystrophy. No. 124 showed gross symptoms at 50 days. A single 15 mg dose of alpha-tocopherol administered on the 56th day resulted in the disappearance of symptoms and a rapid gain in weight. On the 98th day the animal succumbed to its second attack of the disease. The other vitamin E-deficient animals were not supplemented and either died or were killed when moribund after 49 to 79 days on the diet. Throughout the last month of the experiment the food consumption of controls Nos. 123 and 127 was paired with E-deficient rabbits Nos. 122 and 126. At autopsy the remaining testis was removed and weighed, a smear of the epididymis examined for motile sperm, and sections prepared of the testis, epididymis and thigh muscles. The weight changes and degree of muscle damage are given in Table I. Although extensive, the muscle lesions did not quantitatively equal those generally found in young dystrophic rabbits.[5, 6]

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[5] Mackenzie, C. G., and McCollum, E. V., *J. Nutrition,* 1940, **19**, 345.

[6] Mackenzie, C. G., Levine, M. D., and McCollum, E. V., *J. Nutrition,* 1940, **20**, 399.

[7] Mason, K. E., *Am. J. Anat.,* 1933, **52**, 153.

[†] Kindly supplied by Merck & Company, Inc.

TABLE I.
Weight Changes and Muscle Lesions in Male Rabbits Maintained on a Vitamin E-deficient Diet with and without the Addition of Various Amounts of Alpha-tocopherol.

Rabbit No.	Alpha-tocopherol* mg	Initial wt, g	Max. wt, g	Time of max. wt, days,	Final wt, g	Duration of exper., days	Microscopic muscle lesions
120	5	2380	2390	7	1570	22	—
121		1990	2050	41	1720	49	+±
123	5	2400	2450	70	2250	75	—
122		2130	2220	69	1940	73	++
127	5	2240	2400	77	2280	79	—
126		2280	2320	66	1830	79	+±
124		2080	2200	83	1890	98	++
50	3	400	2540	168	2540	168	—
22B	1	470	2310	126	2310	126	±
72	0.5	420	3170	140	3170	140	+±
74	†	370	2720	194	2220	234	+±

*Alpha-tocopherol given 6 days a week.
†See text for levels of alpha-tocopherol administered.

In addition to this group of adult animals, 4 immature rabbits were fed diet 13 plus varying levels of alpha-tocopherol (administered 6 days a week). Rabbit No. 50, receiving a 3 mg level of the vitamin, showed no symptoms or muscle lesions after 168 days on the diet.

Rabbit No. 22B was fed alpha-tocopherol at a 1 mg level. Daily creatine determinations initiated at the eightieth day revealed a slight but definitely abnormal creatinuria. When the animal was sacrificed at 126 days the creatinuria had subsided, and only 1 or 2 muscle lesions were present in each section of thigh muscle. The vitamin E intake was evidently extremely near the minimum antidystrophy level for this animal.

The daily creatine excretion of No. 72 (0.5 mg level of alpha-tocopherol), determined from the 88th to the 105th day, was found to average 45 mg as compared to the normal level of 10 mg or less.

Rabbit No. 74 (0.5 mg level of alpha-tocopherol) excreted an average of 30 mg of creatine daily from the 50th to the 82nd day, at which time the daily dose of alpha-tocopherol was increased to 3 mg. On the 95th day the dose was dropped to 0.3 mg and on the 180th day it was discontinued altogether. The maximum weight was attained on the 194th day; thereafter the animal gradually lost weight until the 233rd day, when it developed physical symptoms of dystrophy and was killed. Animal No. 74, therefore, represents a chronic case of dystrophy terminating in a more acute phase of the disease.

The duration of the experiment, weight changes and muscle lesions for these animals are recorded in Table I.

The testes from both the chronic and acute deficiency groups, with the exception of those from 3 animals, were normal. The second testis of rabbit No. 121 showed some indication of disorganization. The epididymis, on the other hand, contained but a few scattered germ cells, mixed with an abundance of spermatozoa. There is a possibility of mild injury in this case. The second testis of rabbit No. 124 showed sloughing and degeneration of germ cells. The ducts of the epididymis, particularly the proximal ones, contained germ cells of all types mixed with spermatozoa. In the testes of rabbit No. 72 a small number of germ cells were either being sloughed or degenerating *in situ*. Only a few germ cells were found in the epididymides. In all 3 of these cases many tubules showed normal spermatogenesis, and in none of them did the alterations closely resemble those occurring in the testes of vitamin E-deficient rats.

The testes and the contents of the epididymides of the remaining animals of both groups were normal. In all animals, including the 3 discussed above, motile sperm were found in the epididymides at autopsy. The ratio of gram testis weight to kilogram body weight determined at the beginning and end of the experiment on the group of unilaterally castrated animals showed no significant change, the average ratios being 1.12 and 1.23 respectively.

A specific effect of vitamin E on the testes of the adult rabbit is not excluded by these experiments. Prolonging either the chronic or acute deficiency might reveal such an effect. In the latter case inanition and respiratory infections would have to be eliminated as contributing factors. The results do indicate, however, that on the E-deficient diet employed the rabbit's skeletal muscles are more susceptible to pathological alterations than the testes. Muscle necrosis can precede testicular damage and be prevalent in its absence. This is in sharp contrast to the early development of testicular degeneration in rats reared on E-deficient diets. Whether or not there is always a concomitant necrosis of occasional muscle fibres in this species is not known.

Normal testis structure (and probably function) in the rabbit is no more a criterion of adequate E intake than are continued growth, good appetite, and normal appearance.[6] Urinary creatine determinations and histological examination of the skeletal muscles may reveal the existence of an unsuspected chronic vitamin E deficiency in apparently normal animals.

Conclusions. In contradistinction to the rat, the skeletal muscles of the male rabbit with a partial or complete dietary deficiency of vitamin E are, under the conditions employed, more sensitive to morphological alterations than the testes. The absence of testicular degeneration in the rabbit does not preclude the existence of vitamin E deficiency and necrosis of the skeletal muscles.

13069

Tocopherol Level in Serum of Normals and Patients with Amyotrophic Lateral Sclerosis.*

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Synthetic vitamin E (dl-α-tocopherol) has recently been introduced in the treatment of amyotrophic lateral sclerosis.[1] It seemed of interest to study the level of this vitamin in untreated and treated patients with this condition, and to compare it with the serum level of normal individuals. Analytical methods for the determination of tocopherol have been developed for the control of preparation and concentration of this substance from its natural sources, such as wheat germ oil. The dipyridyl method, based on the reduction of trivalent iron by tocopherol to the bivalent state and subsequent colorimetric determination of the ferrous iron as α,α'-dipyridyl complex, has been developed for the assay of tocopherol concentrates. It has been used for the study of the tocopherol level in the serum of tocopherol-deficient and tocopherol-treated rats.[2]

We have adapted this method to the analysis of human serum by means of a photoelectric colorimeter. The employment of a photoelectric method is advantageous because the pink color of the iron-dipyridyl complex is not very suitable for visual comparison.[†] Ten ml of serum from a fasting blood specimen are sufficient for the determination.

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* This work was carried out under grants from the John and Mary Markle Foundation, New York, and the Hoffmann La Roche Company, Nutley, New Jersey. We hereby gratefully acknowledge their generous aid.

[1] Wechsler, I. S., *J. A. M. A.*, 1940, **114**, 948; *Am. J. Med. Sci.*, 1940, **200**, 765; *Arch. Neurol. Psych.*, 1941, in press.

[2] Emmerie, A., and Engel, C., *Rec. Trav. Chim. des Pays-Bas*, 1938, **57**, 1351; 1939, **58**, 283, 895; *Vitamin E, A Symposium*, London, 1939, p. 14.

[†] The details of this method will be reported elsewhere.

Results. We have determined the tocopherol level in the serum of 12 normal subjects. This group comprised 9 men and 3 women in the age group from 23 to 30. The values vary between 0.59 and 1.62 mg/100 ml with an average of 0.96 mg/100 ml, the mode being 0.895. The highest values may be due to the ingestion of food especially rich in tocopherol, and we surmise that individuals on a diet with maintenance amounts of vitamin E display values of 0.6-1.0 mg %. No values for normal animals or men have come to our attention in the literature. Emmerie and Engel[2] give the values 0.064 and 0.056 mg % for the serum of deficient rats, and from 0.29 up to 1.10 mg % for the same animals upon ingestion of 1 mg tocopherol per diem, corresponding to a dose of 250 mg p.d. for a 50 kg human subject. In the organs of rats Karrer, Jaeger and Keller found tocopherol contents in the same range, namely 0.2-1.5 mg/100 g;[3] in Jensen sarcoma Euler and Euler found 0.92 mg/100 g.[4]

One female subject with duodenal ulcers, who was on a restricted diet and who is not included in the above group, showed the low values 0.39 and 0.58% at two different occasions. The lowest value (0.59 mg %) in the above series was obtained on a subject with a one-sided meat and protein diet, who also showed subnormal vitamin A and carotene values in his serum.

We have carried out 33 determinations on 13 patients with amyotrophic lateral sclerosis. These analyses are summarized in

TABLE I.
Level of dl-α-Tocopherol in Normals.

Name	Age	Sex	Vitamin E, mg in 100 cc serum
1. M.S.	26	m	.59
2. H.J.	25	m	.60
3. A.S.	28	m	.60
4. M.E.	30	m	.73
5. F.R.	22	f	.84 .90
6. J.L.	23	f	.89
7. E.W.*	29	m	.89
8. F.S.	26	f	.93
9. M.S.	24	m	1.13 1.62
10. R.L.	25	m	1.24
11. E.K.	25	m	1.43
12. A.L.	28	m	1.43

*This subject, after 7 daily oral doses of 740 mg vitamin E, showed a blood level of 1.50 mg%.

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[3] Karrer, P., Jaeger, W., and Keller, H., *Helv. Chim. Acta,* 1940, **23**, 464.

[4] v. Euler, B., and H., *Z. physiol. Chem.,* 1940, **265**, 147.

Table II. Four of these patients had been admitted without having previously received tocopherol treatment, and their serum values from 0.52-1.00 (average 0.76) mg % fall well within the limits of our normal controls. The majority, however, had received vitamin E treatment of one form or another before serum could be obtained. The treatment as far as it was administered in the hospital consisted of oral administration of Ephynal (Hoffmann-LaRoche), the acetate of dl-α-tocopherol. The daily and total amounts are given in the fourth column of the table. Some cases received from 100 to 200 mg α-tocopherol in sesame oil by intramuscular injections. All patients received in addition up to 2 ounces of wheat germ. A few were given 6 capsules Tocopherex (Squibb) per diem. The values during treatment vary from 1.13 to 2.26 mg tocopherol per 100 ml serum. Values around the 2 mg % level are obtained even with the smallest dosages used; hence it seems that an upper threshold obtains at 2.0-2.5 mg %, which cannot be surpassed even by the administration of liberal amounts. A normal control with 0.89 mg % showed a rise to 1.50 mg % upon daily ingestion of 740 mg of Ephynal during 6 days.

Upon discontinuation of vitamin E treatment, the blood level showed a downward trend, and the figures "off treatment" vary from the upper limit reached in the course of treatment, to low normals.

TABLE II.
Level of dl-α-Tocopherol in Patients with Amyotrophic Lateral Sclerosis.

No.	Age	Sex	Mg% tocopherol before treatment	Daily dosage in mg		Mg% tocopherol during treatment	Mg% tocopherol after treatment was interrupted for No. of days given	Mg% tocopherol on resumption of treatment
				Oral	Intramusc.			
1.	48	f	—	740	200	1.71	—	—
2.	41	m	—	740	200	1.68	—	—
3.	32	m	—	740	200	2.07	1.37 (7 days)	—
4.	58	f	—	100?	—	1.13	—	—
5.	47	m	0.69	150	—	---	—	—
6.	39	f	0.80	340	—	2.26	2.47 (2 days) 1.01 (5 days)	1.36 (3 days)
7.	58	f	—	740	200	1.79	—	—
8.	39	m	—	740	200	2.14	1.36 (4 days) 1.15 (6 days)	1.36 (1 dose of 50 mg oral)
9.	50	f	—	100	—	1.20	0.89 (6 days)	—
				100	—	1.16	0.61 (9 days)	
				—	100	0.90	—	—
10.	43	m	0.55	340	100	0.93	—	—
			0.52	—	—	--		
11.	50	m	0.69	740	—	1.70	—	0.61 (3 doses of 200 mg intramusc.)
			0.88	740	—	2.00	—	
12.	48	m	0.96	—	100	0.59	—	—
13.	38	m	1.00	—	100	0.67	—	—
				100	—	0.90		

Conclusion. From a clinical study which showed definite improvement in some cases following the administration of vitamin E, the conclusion seems warranted that the vitamin brought the results. But the above figures, although comparatively few in number, do not point to a simple vitamin E deficiency in amyotrophic lateral sclerosis. They show that the vitamin E level in the serum of untreated patients falls within the normal range. The response of the blood level to oral administration indicates that substantial amounts of 50-100 mg easily enter the blood stream. As it is known that no vitamin E is deposited in the organs of animals, it is unlikely that it accumulates in human organs. Hence, one cannot state whether the administered synthetic vitamin is excreted, destroyed or deposited as far as it is not accounted for in the circulation.

A comparison with vitamin A studies shows the following: When 100,000 I.U. of vitamin A are given, only a small quantity, never more than 3%, is recovered in the blood.[5] This vitamin is known to be stored in the liver; moreover, it is chemically less stable than tocopherol. Thus, if similar quantities of the latter are administered, a very much larger proportion appears in the circulation.

We do not know how and where vitamin E exerts its effect on nerve and muscle function. It may be that there is a barrier to the passage of tocopherol from the blood stream to nerve and muscle cells in amyotrophic lateral sclerosis, or that another obstacle of a functional nature prevents the utilization of the vitamin found in the blood at its normal level.

Patients stated that they felt better or stronger on treatment, but such subjective improvements are difficult to evaluate. Two patients in the present series showed definite objective improvement, and a third an apparent improvement, with the rise of the tocopherol level in the blood. The rest did not, and yet all responded to oral administration with an increase of serum tocopherol. This increase did not parallel the dosage ingested. That is, after 50 or 100 mg had been given, it did not matter whether the dosage was increased even to 500 mg. It is probable that the tocopherol content of the blood takes at least two or three days before reaching the maximum level; on the other hand, this level persists for a few days after administration has been stopped. In three patients who received intramuscular treatment without simultaneous oral treatment, a drop of the blood tocopherol level may be noted in the table.

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[5] Peck, S. M., Chargin, L., and Sobotka, H., *Arch. Derm. Syph.*, 1941, **43**, 223; unpublished observations of Brickner, R., Kann, S., and Sobotka, H.

We wish to thank Drs. M. Ellenberg, S. Margolin and M. Sapirstein for their kind assistance with the patients.

Summary. (1) Normal serum tocopherol values determined by a new photocolorimetric method are given. (2) Untreated patients with amyotrophic lateral sclerosis show values within the normal range. (3) The blood level invariably rises on oral administration, irrespective of whether the patient does or does not show clinical improvement. (4) Intramuscular administration without simultaneous oral administration produces a drop in serum tocopherol.

13070

Comparison of Effects of Large Doses of Various Activated Sterols on Serum Calcium.

EVAN W. MCCHESNEY AND HUGO KOCHER. (Introduced by O. W. Barlow.)

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When highly potent preparations of vitamin D became available, there was an immediate interest in the physiological effects of massive doses of the vitamin, and numerous studies dealing with their effects on the metabolism of calcium and phosphorus were reported. The literature has been reviewed by Schmidt and Greenberg,[1] and more recently by Reed, Struck and Steck,[2] therefore requires no extensive comment here. In general, it has been established that in normal animals large doses of vitamin D_2 result in a decreased fecal excretion of calcium, a hypercalcemia, and an increase in urinary calcium and phosphorus.

Other activated sterols might be expected to exert similar effects. Among these are vitamin D_3, dihydrotachysterol (A.T.10), and "Ertron". The last named is a form of activated ergosterol produced by the Whittier process and claimed by its manufacturers* not to have the hypercalcemic or other toxic effects of calciferol.[3]

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[1] Schmidt, C. L. A., and Greenberg, D. M., *Physiol. Rev.*, 1935, **15**, 297.

[2] Reed, C. I., Struck, H. C., and Steck, I. E., *Vitamin D: Chemistry, Physiology, Pharmacology, Pathology, Experimental and Clinical Investigations,* Univ. of Chicago Press, 1939, p. 219.

* Nutrition Research Laboratories, Chicago, Ill.

[3] *Cf.* also: Snyder, R. G., and Squires, W. H., *N. Y. State J. Med.*, 1940, **40**, 708.

Vitamin D_3 and A.T.10 have not been studied extensively experimentally probably because they have not been generally available. The literature on these preparations up to 1939 is included in the review of Reed, Struck and Steck. More recently Weber and Richardson[4] have shown that in human subjects A.T.10 increases serum calcium and increases the absorption of calcium from the gut. Shohl and Farber[5] have shown that A.T.10 exerts a hypercalcemic effect in rats considerably greater than would be predicted from its antirachitic value.

It is our purpose in this paper to present a comparative study of the effects on serum calcium of various amounts of the activated sterols mentioned, the doses being chosen in such a way as to remain within physiological limits. For test animals we selected adult albino rats from our laboratory stock colony; they varied from 175 to 240 g, and 90 to 120 days of age. They received, *ad libitum,* the regular breeding diet and water. The various preparations were administered by stomach tube as a single dose in 0.5 cc of peanut oil, and the animals were sacrificed by decapitation at the indicated intervals after medication. The blood was collected in centrifuge tubes, allowed to clot, and then centrifuged. Serum calcium was determined on each animal by the Clark and Collip[6] modification of the Kramer and Tisdall[7] method. The test groups consisted of at least 7 animals, and in some cases 10. A group of 34 control animals run at various times during the tests gave the average value for serum calcium of 11.15 ± 0.35[†] mg %. Schmidt and Greenberg[8] have reported this value as 11.3 ± 0.50[†] mg %.

In one experiment doses of 25,000 I.U. of vitamins D_2, D_3 and of Ertron were given, the animals being sacrificed in groups at daily intervals up to 4 days. Since this treatment did not result in a hypercalcemia of the degree desired, we carried out a second experiment in which the doses were increased to 100,000 I.U. of each preparation. In this experiment we also gave 0.5 cc of commercial A.T.10 (0.5% solution), and 0.5 cc of a special A.T.10 (1.0% solution). Groups of animals were sacrificed at regular intervals as long as a significant hypercalcemia persisted. The results of the experiments are shown in Figs. 1 and 2.

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[4] Weber, F. C., and Richardson, H. B., *J. Clin. Endocrinol.*, 1941, **1**, 32.

[5] Shohl, A. T., and Farber, S., *J. Nutrition*, 1941, **21**, 147.

[6] Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, 1925, **63**, 461.

[7] Kramer, B., and Tisdall, F. F., *J. Biol. Chem.*, 1921, **47**, 475.

[†] Standard deviation.

[8] Schmidt, C. L. A., and Greenberg, D. M., ref. 1, p. 334.

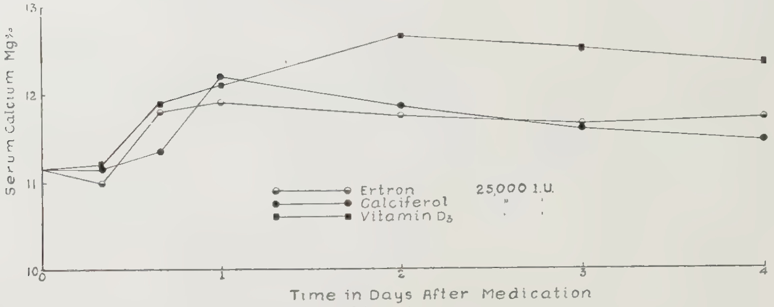

FIG. 1.

Effect of moderate doses (as to hypercalcemia) of vitamin D preparations on the serum calcium of the rat.‡

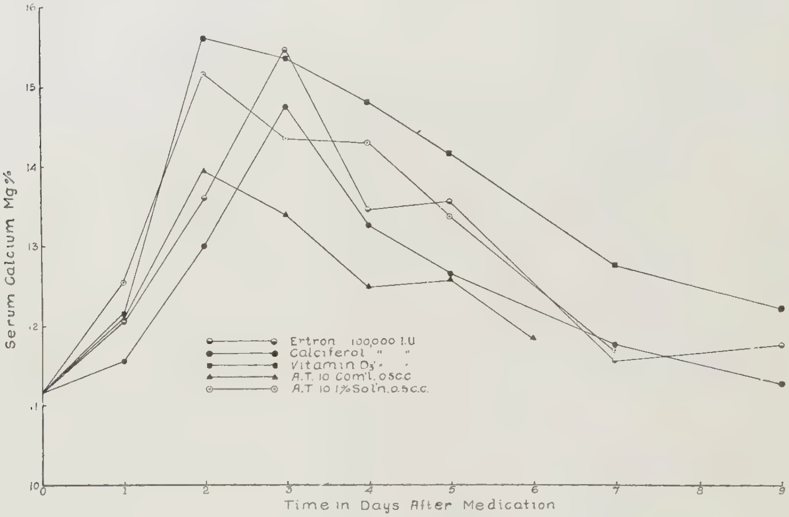

FIG. 2.

Effect of massive doses (as to hypercalcemia) of vitamin D preparations and of A.T.10 on the serum calcium of the rat.

Discussion. The most important conclusion which can be drawn from these experiments is that the effect of vitamin D_3 on serum calcium differs both quantitatively and qualitatively as to the type of curve produced from those of either of the activated ergosterol preparations. In other words, the serum calcium peak value following vitamin D_3 is not only reached at a different time, but the hypercalcemia induced persists for a significantly longer period of time than after activated ergosterol. Thus, the mass effect of vitamin D_3 (*i. e.,* elevation of serum calcium x time) is markedly greater (at least 50% on the 100,000 unit dose) than that of D_2.

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‡ In both Fig. 1 and 2 each point represents the average of at least 7 animals.

A second conclusion is that Ertron and calciferol are indistinguishable in their effects on the serum calcium of the rat when corresponding U.S.P. or international antirachitic vitamin D dosages are administered. In both experiments the curves for the two products are not separated by a statistically significant amount at any point; the probable error calculated for each point is from 0.2 to 0.5 mg %.

The effects of the larger dosage of A.T.10 very closely resemble those of the 100,000 I.U. dosage of vitamin D_3. In terms of antirachitic activity, *i. e.*, vitamin D, the dose of this solution given represents only 22 to 25 I.U. per mg or a total dose of 110 to 125 units. Such a dosage is thus approximately 1/850 of the potency of the vitamin D_3 so that the effects of A.T.10 on serum calcium cannot be attributed primarily to the presence of contaminating antirachitic sterols. Finally, the marked difference between the effects of the two doses of A.T.10 given suggest that the determination of serum calcium might prove to be a satisfactory basis for the bioassay of the product to replace the original "toxic borderline dose" method.

Summary. The effects of vitamins D_2, D_3, and of Ertron and A.T.10 on serum calcium have been compared. Crystalline vitamin D_2 and Ertron are statistically indistinguishable in their effects. Vitamin D_3 produces a more prolonged hypercalcemia than does vitamin D_2. On the basis of responses to the above preparations, A.T.10 produces an effect far greater than would be predicted from its antirachitic value.

13071

Inhibition of Bacteriostatic Action of Sulfanilamide by Yeast Extracts.

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Substances capable of preventing the bacteriostatic action of sulfanilamide have been found widely distributed in nature.[1] Woods[2] reported that a substance was present in an extract prepared from Baker's yeast which lessened the inhibitory effect which sulfanilamide has upon the growth of *Streptococcus hemolyticus*. Further experiments upon the properties of this compound led him to the conclusion that it resembled p-aminobenzoic acid, and investigations of preparations of that substance in pure solution showed that it markedly inhibited the anti-bacterial action of sulfanilamide. Since this paper was published, Rubbo and Gillespie[3] have isolated p-aminobenzoic acid as the benzoyl derivative from a Brewer's yeast extract. *In vivo,* p-aminobenzoic acid prevents the favorable action of sulfanilamide upon experimental infections with streptococci and pneumococci as shown by Selbie[4] and McCarty.[5]

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[1] MacLeod, C. M., *J. Exp. Med.*, 1940, **72**, 217.

[2] Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.

Woods[2] found that his extracts contained, in addition to the anti-sulfanilamide factor, a compound which promoted the growth of the organisms studied. This substance was present in small amounts because after dilution of these extracts its presence could no longer be demonstrated, although the anti-sulfanilamide action could still be shown. It is generally agreed that the bacteriostatic action of sulfanilamide is influenced by the composition of the culture medium, particularly by peptone and other growth-promoting factors.[6, 7] Therefore, at the present time anti-sulfanilamide activity appears to be due to (a) factors that are favorable to the growth of the organisms and (b) compounds that specifically interfere with the action of sulfanilamide.

In this paper experiments designed to extend further the study of anti-sulfanilamide activity of yeast extracts are reported. Attempts were made to prepare extracts from Baker's yeast in which substances favoring the growth of organisms would be absent.

A fat-free, protein and peptone-free, extract from Baker's yeast was made by a technic similar to that developed by Stamp[8] and Woods.[2] Eight different samples of yeast were used, each weighing approximately 250 g. The procedure involved extraction with ammonia, precipitation with phosphotungstic acid, removal of excess phosphotungstic acid with barium hydroxide, and concentration at reduced pressure at a pH of approximately 7.0. The extract was then treated with 10 volumes of acetone because it was thought that the growth-promoting property of the extracts may be due to the presence of complex carbohydrates. A precipitate was obtained which was removed by centrifuging the solution, and the treatment with acetone was repeated twice. Acetone was removed from the supernatant fluid by evaporation to dryness. This dry residue was dissolved in distilled water, the pH adjusted to 7.0, and dilution was made so that 1 cc was equivalent to 1 g of the original yeast cells. This solution will be referred to hereafter as extract A. This preparation gave a positive reaction for reducing compounds (3 to 5 mg % as dextrose) by the method of Klendshoj and Hubbard,[9] but no fermentable sugar was present. No significant increase in reducing action was obtained after acid hydrolysis. When treated by the diazo reaction of Bratton and Marshall,[10] its content of p-aminobenzoic acid appeared to be 0.06 mg %. The precipitate obtained from the action of acetone was dried, dissolved in distilled water to give 1 cc equivalent to 1 g of the yeast cells, and the solution adjusted to a pH of 7.0. This solution, which is referred to as extract B, gave a negative reaction for starch with iodine. It contained a small amount of reducing material (15 to 20 mg %) the larger proportion of which was fermentable by treatment with yeast. Acid hydrolysis yielded small additional amounts of reducing compounds (5 to 10 mg %). No reaction was obtained by the diazo technic of Bratton and Marshall.[10]

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[3] Rubbo, S. D., and Gillespie, J. M., *Nature,* 1940, **146**, 838.

[4] Selbie, F. R., *Brit. J. Exp. Path.,* 1940, **21**, 90.

[5] McCarty, M., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 61.

[6] Long, P. H., and Bliss, E. A., *So. Med. J.,* 1938, **31**, 308.

[7] Lockwood, J. S., and Lynch, H. M., *J. Am. Med. Assn.,* 1940, **114**, 835.

[8] Stamp, T. C., *Lancet,* 1939, **2**, 10.

The bacteriological tests were carried out *in vitro* on strains of beta *hemolytic streptococci* (group A Lancefield) and *E. coli*. In tests with the former organisms the solution to be tested was added to 1% dextrose phenol red broth (Difco) containing sulfanilamide in varying concentrations. In tests with *E. coli* the synthetic medium of Sahyun, Beard, *et al.*,[11] which was free of demonstrable antisulfanilamide activity was prepared in a similar manner. The mediums were sterilized in test tubes by autoclaving at 15 lb for 12 minutes. Organisms freed from nutrient material by washing and resuspending in physiological saline were used for seeding purposes. Inoculated material was incubated at 37°C. Visible growth of the organisms in the material tested was taken as evidence of the presence of growth factors. Visible growth in the medium containing the test material and sulfanilamide, in contrast to the absence of visible growth in the sulfanilamide control indicated antisulfanilamide activity.

The results of these tests showed that both extract A and extract

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[9] Klendshoj, N. C., and Hubbard, R. S., *J. Lab. Clin. Med.*, 1940, **25**, 1102.

[10] Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, **128**, 537.

[11] Sahyun, M., Beard, P., Schultz, E. W., Snow, J., and Cross, E., *J. Inf. Dis.*, 1936, **58**, 28.

B inhibited the bacteriostatic action of sulfanilamide toward *hemolytic streptococci* as well as *E. coli.* Both preparations also promoted growth of *E. coli* under these experimental conditions. Because there is evidence that the action of these yeast extracts may be due to p-aminobenzoic acid, a comparative study of the anti-sulfanilamide activity of these extracts and p-aminobenzoic acid was carried out. p-aminobenzoic acid showed anti-sulfanilamide activity when tested in concentrations approximating those found in extract A. It did not promote growth of *E. coli,* although in higher concentrations (10 to 100 mg %) it prolonged the survival of this organism. p-aminobenzoic acid was extracted from acid aqueous solutions by ether, and the anti-sulfanilamide activity was destroyed by acetylation as carried out by the method of Woods[2] but was subsequently recovered on acid hydrolysis.

After extraction with ether from an acid medium or acetylation of extract A, the diazo reaction became negative, but only part of the anti-sulfanilamide activity was lost. On subsequent hydrolysis following acetylation the extract did not recover demonstrable anti-sulfanilamide activity, although the amino compound was regenerated as shown by the development of a positive diazo reaction. In the ether-soluble portion of extract A which was evaporated to dryness and taken up in water, anti-sulfanilamide activity could be demonstrated as well as a positive diazo reaction, and this ether-soluble portion was not growth-promoting to *E. coli.* Thus, the ether extracted and acetylated anti-sulfanilamide factor in extract A may be p-aminobenzoic acid. Extract A showed a much greater anti-sulfanilamide activity than did the presumably equivalent solution of p-aminobenzoic acid. In extract A, therefore, there was apparently not only p-aminobenzoic acid but also another factor which diminished the bacteriostatic action of sulfanilamide. The results of the study of extract B were in some ways similar to those obtained with extract A. As in extract A, acetylation procedures destroyed part of the anti-sulfanilamide activity and subsequent hydrolysis completely destroyed this property of extract B. However, unlike extract A, growth-promoting properties of extract B were partially recovered by acid hydrolysis. Extract B did not contain p-aminobenzoic acid as shown by a negative diazo reaction and negative anti-sulfanilamide activity of an ether extract prepared from it. The anti-sulfanilamide activity and the growth-promoting properties of the extract persisted apparently unchanged after ether extraction. The results of these experiments are summarized in Table I.

In conclusion, the anti-sulfanilamide activity of the yeast ex-

TABLE I.
Effect of Ether Extraction and Acetylation on Activity of Para-aminobenzoic Acid and Yeast Extracts.

	Anti-sulf. activity	Growth-promoting activity	Diazo test
1. p-aminobenzoic acid (100 mg%)			
natural	+	—	+
treated with ether	—		—
ether soluble fraction	+		+
acetylated	—	—	—
acetylated then hydrolysed	+	—	+
2. Extract A			
natural	+	+	+
treated with ether	+	+	—
ether soluble fraction	+	—	+
acetylated	+	—	—
” then hydrolysed	—	—	+
natural extract hydrolysed	+	+	+
3. Extract B			
natural	+	+	—
treated with ether	+	+	—
ether soluble fraction	—	—	—
acetylated	+	—	
” then hydrolysed	—	+	
natural extract hydrolysed	+	+	—

+ = Test is positive or activity is present.
— = ” ” negative ” ” ” absent.

tracts was due to at least two factors. One has properties which closely resemble p-aminobenzoic acid and the other factor differs from p-aminobenzoic acid because anti-sulfanilamide activity persists after ether extraction and acetylation procedures which destroy the anti-sulfanilamide activity of p-aminobenzoic acid. The experiments suggest that this second factor is possibly made up of two different compounds, one soluble and the other insoluble in acetone. The experiments further showed no absolute relationship between growth-promoting properties and anti-sulfanilamide activity of these extracts, since fractions of yeast extracts were prepared which promoted the growth of *E. coli* without showing demonstrable anti-sulfanilamide activity, and conversely, fractions which had anti-sulfanilamide activity without promoting growth of the organisms.

13072 P

Treatment of Infected Wounds in the Hamster by Contact Application of Ultraviolet Rays.

Ralph Herz.* (Introduced by W. E. Hambourger.)

With the hope of improving the methods for the treatment of infected wounds in surgical practice, a series of animal studies was undertaken with the Gosztonyi lamp. This instrument is designed to yield the maximum output of radiation in the range of short ultraviolet waves. It is claimed that 93% of the output of ultraviolet radiation is of a wavelength of 2537 Angstrom units, which is the optimum wavelength for bactericidal action as determined by various investigators. With the Gosztonyi instrument the ultraviolet radiation is delivered through small cold quartz applicators of various shapes and sizes which facilitate the application of the radiation by direct contact. This has an important advantage in the irradiation of infected cavities and wounds, because the efficacy of irradiation varies according to the inverse square law, that is, the quantity of radiation delivered to a given site is inversely proportional to the square of the distance from the target for a point source. Another claim made for this instrument which has been corroborated in these studies is that no heat is generated in the applicator. The instrument was tested repeatedly throughout these studies and its temperature never varied from room temperature more than 3°C.

Before attempting to use this instrument in the treatment of patients, preliminary experimental studies were carried out on animals. As a first step in the investigation, the bactericidal efficacy of the instrument was tested by irradiation of cultures of colon bacillus, *Staphylococcus aureus,* and the Hay bacillus prepared on agar plates. Then the direct application of ultraviolet radiation to infected wounds by means of the Gosztonyi apparatus was carried out on a group of Syrian or golden hamsters. Fifty-five animals were selected for the experiments, and the studies were carried to completion on 44. Eleven animals were sacrificed to the preliminary attempts to determine the proper dosage of anesthetic and the most satisfactory organism for the production of infected wounds. Three died from the anesthetic, one from peritonitis, and 7 had unsatisfactory and irregular abscess formation produced by cultures of *Escherichia coli.* In these preliminary trials it was found that the most satisfactory lesions were produced with a virulent strain of *Staphylococcus aureus,* and this organism was used in the 44 animals on which the studies were completed.

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The animals were anesthetized with 0.2 cc of a 1% solution of nembutal, injected intraäbdominally. A pure culture of the *Staphylococcus aureus* on slant agar in a 10 cc test tube was diluted with broth to a sufficient quantity to inoculate 6 animals at a time. An injection of 0.2 cc of this dilution was made over each scapula of the hamster. Following the inoculation, the left side was incised and sutured. A similar procedure was carried out on the right side, except that the wound was irradiated by direct contact with the ultraviolet applicator for 5 to 15 minutes before it was sutured. With this technic, each animal served as its own control. The time which elapsed from the injection of the culture to irradiation varied from 5 to 90 minutes.

Of the 44 animals inoculated with a virulent culture of *Staphylococcus aureus,* an abscess developed on the left side, which was not irradiated, in 37 animals; in 7 animals there was no abscess on the non-irradiated side. On the right side, which had been irradiated, there was no abscess in 35 animals; 9 showed abscess formation. Seven of the 9 animals in which an abscess developed on the irradiated side were among the first injected, and it was in this group that the irradiation period was shorter and the time between inoculation and irradiation longer.

Ten of the animals in which an abscess developed were selected for another study to test the efficacy of the ultraviolet applicator in the healing of these lesions. The abscess was opened and drained in each instance, and the applicator was inserted directly into the wound, in 5 cases. In the other 5, no radiation was administered. In the 5 animals thus irradiated, the wounds showed evidence of healing in 24 to 48 hours. In the control series, one abscess healed in 5 days; the others did not heal.

Results. The results of these experimental studies, which proved the bactericidal action of the Gosztonyi applicator and its effectiveness both in prophylaxis and treatment of abscesses produced by *Staphylococcus aureus* furnished the experimental basis for clinical studies.

13073 P

Parthenogenetic Stimulation of Aged Anuran Eggs.

Anita Zorzoli and Roberts Rugh.

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If eggs are retained in the uterus of the frog over an extended period so that insemination is delayed, it is known that radical changes take place in the cortex of such eggs[1] and very likely also in the second polar body elimination.[2] Since the region of activation is the cortex and survival of parthenogenetically stimulated eggs up to and beyond the tadpole stage may be dependent upon the diploid condition (polar body retention?),[3] a significant difference in parthenogenetic data from aged eggs should be noted. A total number of 1807 eggs of progressively increasing ages were stimulated artificially and the incidence of cleavage and development compared with that of a similar number of control eggs which were less than one day old.

Hibernating females *(Rana pipiens)* were induced to ovulate[4] and then kept in a refrigerator at 10°C for the duration of the experiment. On the 1st, 3rd, 5th, 7th, and 8th days eggs were stripped in 2 single rows onto glass microscope slides, pricked in the animal hemisphere with a fine glass needle and allowed to develop in spring water at 17°C. Seven hours after stimulation the eggs were examined and classified as showing surface furrows, deep furrows, and abnormal or normal cleavage. Controls consisted of normally inseminated aged and fresh eggs and pricked fresh eggs.

The data for 7- and 8-day-old eggs only and control 1-day-eggs are given in Table I. It is obvious that not only is there an increase in the total number of eggs showing deep furrows and abnormal cleavage but also a definite increase in the amount of normal cleavage. This difference between aged and fresh eggs disappears at the time of gastrulation. In fact, relative to the number of eggs initially undergoing normal cleavage, there is a smaller percentage of aged eggs which survive gastrulation.

As mentioned before, aging radically affects the integrity of the egg cortex. Zimmerman and Rugh[1] observed changes in permea-

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[1] Zimmerman, L., and Rugh, R., *J. Morph.*, 1941, **68**, 329.

[2] Bataillon, E., and Tchou Su, *C. R. Acad. Sci.*, 1932, **195**, 353.

[3] Parmenter, C. L., *J. Exp. Zool.*, 1933, **66**, 409.

[4] Rugh, R., *Biol. Bull.*, 1934, **66**, 22.

TABLE I.

Frog	Age of eggs	No. of eggs	% surface furrows	% deep furrows	% abnorm. cleav.	% normal cleav.	% gastrulating	% hatching
Control	1	267	20	4	0.8	1.6	.1	.0
A_1	7	211	42	52	1.4	2.8	1.8	1.4
A_2	7	218	44	50	4.6	3.2	0.2	.0
A_3	7	232	10	70	9.0	9.5	0.9	.3
Control	1	260	17	18	0.8	1.9	1.6	.1
A_1	8	30	43	53	0.0	3.3	0.0	.0
A_2	8	84	37	56	3.6	2.8	0.0	.0
A_3	8	100	25	59	5.0	8.0	2.0	.0

Abnormal cleavage—cell stages recognizable but cells not in normal relationship.
Normal cleavage—normal cell relationships and size.

bility, pigment distribution and cytoplasmic vacuolation with increasing age of *Rana pipiens* eggs. To account for the higher incidence of abnormal and normal cleavage in this experiment it might be conjectured that these cortical changes are responsible for a greater sensitivity to stimulation and resultant cleavage. In this connection it is interesting to note that after normal insemination of 7- and 8-day-old eggs, a high percent of the eggs failing to undergo development showed surface and deep furrowing similar to and in some cases identical with that found in the parthenogenetically stimulated eggs. However, as the eggs reach the critical time of gastrulation the derangement of cytoplasmic materials may be sufficient to impede the extensive movements of materials which normally occur. Embryos which went through this stage often showed signs of imperfect gastrulation such as: microcephaly and persistent yolk plug. On the other hand, some cases did develop normally beyond gastrulation. It might be that these resulted from eggs which retained the second polar body as a consequence of aging. It is evident that polar body and early cleavage history studies as well as chromosome counts must be made for aged eggs as were made by Parmenter[5] for normal eggs.

Summary. Aged eggs of *Rana pipiens,* parthenogenetically stimulated, showed a higher percentage of cleavage but a proportionately lower incidence of gastrulation than normal eggs similarly treated.

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[5] Parmenter, C. L., *J. Morph.*, 1940, **66**, 241.

13074

Effect of Diet on Anaphylaxis in the White Rat.

JULIA T. WELD AND LUCY C. MITCHELL.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.

Parker* and Parker[1] reported that they were able to produce anaphylaxis in the white rat, but were unable to explain their success when preceding workers had reported failures.[2] The following year Wedgewood and Grant[3] reported that they were unable to sensitize normal rats to foreign proteins when fed the regular diet, but succeeded when the animals were fed on a diet deficient in vitamin B. These observations were confirmed by Seegal and Khorazo.[4]

In view of the contradictory results obtained we wish at this time to report the results of some investigations planned to learn more about the influence of certain dietary deficiencies on the production of anaphylaxis in the white rat.

Experimental. In their early work Parker and Parker were unable to sensitize young rats weighing 50-75 g, so in the present study we used rats which at the beginning of the experiments were approximately 7 weeks of age and weighed from 130-160 g. Thus at the time the intravenous shocking doses were given the rats were 10 or 11 weeks old. They were placed in cages, 5 in each, and were fed 5 weeks on the diets described in Table I. In each cage there was one control on the same diet which did not receive the sensitizing dose of antigen.

Three sensitizing inoculations of either horse serum or sheep serum† were given intraperitoneally with intervals of one week between. Then one or two shocking doses of the specific antigen were injected intravenously in a tail vein one week and 2 weeks after the last intraperitoneal inoculation. In the table the results are given for only the final intravenous inoculation. In the beginning 1 cc of serum was given both for the sensitizing and the shocking

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*Julia T. Parker, now Julia T. Weld.

[1] Parker, J. T., and Parker, F., *J. Med. Research,* 1924, **44**, 263.

[2] Trommsdorf, R., *Arb. a. d. k. Gendhtsamte,* 1909, **32**, 560; Novy, G. G., and DeKruif, P. H., *J. Infect. Dis.,* 1917, **20**, 776; Longcope, W. T., *J. Exp. Med.,* 1922, **36**, 627.

[3] Wedgewood, P. E., and Grant, H. H., *M. Bull. Univ. of Cincinnati,* 1924, **2**, 172.

[4] Seegal, B. C., and Khorazo, *Arch. Path.,* 1929, **7**, 827.

† Sheep serum was used as sensitizing agent in only the first few experiments.

TABLE I.

Diet No.	Flour	Casein	Cornstarch	Yeast 188	Yeast 20-40	Crisco	Carotene in oil	NaCl	$CaCO_3$	KH_2PO_4	Vitamin D daily*	Rockland rat food	No. of rats on diet	Avg gain	Avg loss	Degree of shock*						Avg Index of degree of shock
																+++++	++++	+++	++	+	0	
1	95.9			.1		2		2					69	5		6	10	27	20	5	1	2.85
2	95.9			.1			2	2					12	20		0	2	8	1	1	0	2.92
3	86				10	2		2					8	53		0	1	4	3	0	0	2.75
4	93.9			.1			2	1	2	1			7	14		0	1	3	3	0	0	3.14
5	75		20.9	.1		2		2					28		7	0	0	2	6	8	12	0.93
6	60		35.9	.1		2		2					16		25	0	0	1	1	1	13	0.38
7	83.9	12		.1		.2		2					25	10		2	2	6	8	5	2	2.29
8		18	77	.1		2		2					46		18	0	0	2	6	5	33	0.5
9		12	83.9	.1		2		2					23		21	0	0	0	3	5	15	0.44
10		18	68		10		2	2					9	33		0	0	4	5	0	0	2.33
11												100	19	71		0	2	5	7	4	1	2.16
12									2			98	8	70		0	0	0	1	4	2	0.75
13											10 units	100	8	53		0	0	2	4	2	0	2.
14									2		10 units	98	8	61		1	1	1	4	1	0	2.62

*+++++ indicates very marked prostration followed by death within 18 hours.
++++ ,, ,, severe symptoms—very marked prostration lasting 4 to 5 hr—appears dying.
+++ ,, severe symptoms— ,, ,, ,, 2 to 3 ,,
++ ,, moderate ,, ,, ,, ,, 1 to 2 ,,
+ ,, slight ,, ,, ,, less than 1 hr.
0 ,, no symptoms.

Flour: Pillsbury's Best XXXX.
Casein: Labco—vitamin-free.
Yeast: Fleischmann's Dried—188.
,, ,, 20-40.
Vitamin A: Carotene in oil, 0.1%; Wesson oil, 1.9%.
,, D: 10 units in 1 cc Wesson oil given by mouth.

doses, but later the sensitizing and the shocking doses were respectively 1 and 1½% of the body weight of the animal. Thus an animal weighing 180 g would receive 1.8 cc serum as a sensitizing dose, and 2.7 cc as a shocking dose. Male rats were used in all experiments except one, in which 20 female rats were used.

Diets. Since we had shown in 1924 that rats on a bread and water diet can be made sensitive to anaphylactic shock, our basic diet in these experiments was composed of the ingredients contained in bread prepared at that time.

A diet made of bread and water is deficient not only in vitamins, but also in some of the essential amino acids and certain minerals; so in these experiments various food substances were substituted for all or part of the flour in the basic diet to see if they would alter the susceptibility of the rats to sensitization. For this purpose there were added to different diets a yeast preparation containing relatively large amounts of the components of the vitamin B complex; carotene in oil to supply vitamin A; casein, to supply the essential amino acids and certain mineral salts to supply those missing from the white flour.

For conciseness we have condensed the data of the various experiments in Table I. The table shows: first, the components of the diets used; second, the number of rats on each diet; third, the average gain or loss in weight; fourth, the degree of shock; and finally, the average degree of shock. The average degree of shock was determined by combining all the plus (+) marks for the rats on one diet and dividing this total by the number of rats inoculated. No distinction is made between the rats shocked with horse serum and those shocked with sheep serum, as the results were similar.

The table shows that the animals will develop anaphylactic reactions if they are in a fair state of nutrition and not losing weight, and that under these conditions the dietary deficiencies studied are unimportant. Apparently the most important factor in sensitizing rats to foreign proteins is age. When rats 11 to 12 weeks of age and of fair nutrition are used, uniformly successful results were obtained. This may explain the failures of others, as we were unsuccessful in producing shock with rats 7 to 8 weeks old.

Summary. It has been found that the addition to the bread diet of yeast, vitamin A, large amounts of casein, or $CaCO_3$ and KH_2PO_4, will not appreciably modify the anaphylactic response in white rats. Apparently rats must be at least 10 weeks of age before they can be used successfully for anaphylactic experiments. Rats poorly nourished to the point where they are losing weight cannot be sensitized to anaphylactic shock.

13075

A Method for the Experimental Production of Brain Abscess.

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The object of this report is to detail a method for the experimental production of brain abscess. The ultimate aim of our work is to evaluate chemotherapy and the various surgical technics in the treatment of brain abscess.

The problem was first attacked by Malinovski.[1] He found that the injection of a virulent culture into the brain would not produce an abscess unless the area had been previously damaged. His technic was to puncture the cortex with a hypodermic needle and then about 10 days later to inject a culture of a virulent organism. Malinovski obtained abscesses in this manner, but he does not give the percentage of success which attended the use of his method.

Essick,[2] working with cats, was able to produce brain abscess by first traumatizing the brain and then inoculating with organisms, preferably obtained from the pus of other cat brain abscesses. However, most of his animals died within 48 hours of an overwhelming toxemia or meningitis. These abscesses, while satisfactory for a pathological study of acute brain abscess, were followed by death of the animal too soon for surgical intervention or for study of the subacute and chronic lesion. Groff[3] listed many of the unsuccessful methods that had been tried in this laboratory previous to that time, and presented a procedure which gave more or less irregular results. He reported abscesses in 5 dogs which survived for from 3 to 8 days.

The following procedure is suggested as one that will produce abscesses uniformly with survival of the animal for a long enough time to allow evaluation of chemotherapy and varying surgical technics in treatment. Because of repeated failure in this and other laboratories uniformly to produce brain abscesses by intracerebral injection of bacteria alone, local tissue injury was first produced by the method to be described.

Procedure. Under general anesthesia 0.5 cc of a 0.3% solution of sodium ricinoleate (Soricin—Merrell) in melted agar is injected subcortically through a small burr hole. With the removal of the skin sutures, on about the fifth day, 0.02 cc of a 12-hour culture of bacteria (Pneumococcus Type III) is injected through the burr hole into the original site of injection of the sodium ricinoleate.

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[1] Malinovski, *Centralblatt f. d. med. Wissenschaften*, Berlin, 1891, **29**, 162.

[2] Essick, C. R., *Arch. Neurol. and Psych.*, 1919, **1**, 671.

[3] Groff, R. A., *Arch. Neurol. and Psych.*, 1934, **31**, 199.

Fundal examinations and cisternal punctures were made previous to the injection of the culture and at varying intervals afterward. The cerebrospinal fluid pressures, cell counts, and any neurological signs were recorded. In most cases the animals were allowed to die and post mortem examinations were made as soon as possible. The brains were placed in a 10% solution of formaldehyde and sectioned later.

The protocols in Table I show the course of a control group of dogs injected with sodium ricinoleate but not inoculated with organisms. Dogs Nos. 244, 126, and 160 were operated upon on 12-9-40. Fundal examinations and spinal fluid studies were carried out on the seventh, sixteenth, twentieth, thirtieth and forty-fifth postoperative days.

Table II tabulates the typical results of this method with inoculation.

It is not the purpose of this paper to detail the pathological process

TABLE I.
Control Dogs Injected with Sodium Ricinoleate Not Followed by Injection of Pneumococci.

Dog	Post-operative day	Spinal fluid pressure	Spinal fluid cells	Remarks
244	7	140	15	Stitches removed, wound satisfactory
	16	140	10	No neurological signs.
	20	140	7	” ” ”
	30	140	8	” ” ”
	45	160	10	
126	7	136	12	Stitches removed, wound satisfactory.
	16	130	12	No neurological signs.
	20	200	12	Dog excited at time. No neurological signs.
	30	180	25	Cannot account for the rise in cells. No neurological signs.
	45	175	740	Cells unaccounted for. Dog does not show any neurological signs; however, it does not appear well.
160	7	160	14	Stitches removed, wound satisfactory.
	16	—	—	
	20	150	5	No neurological signs.
	30	150	7	” ” ”
	45	150	12	

TABLE II.
Dogs Injected with Sodium Ricinoleate and with Pneumococci.

Dog	Days post-operative when inoculated	Spinal fluid pressure		Spinal fluid cell count		†Optic disc after inoculation	Culture of Abscess	Neurological findings	End results
		Before Inoculation	After Inoculation	Before Inoculation	After Inoculation				
171	1	120	200	5	15	Choked 1 diopter	Pneumo. 7th day	Right hemiparesis 3rd day after inoculation	Died 7th day after inoculation
124	9	110	190	27	8	Choked 2 diopters	Pneumo. 13th day	Right hemiparesis 8th day	Sacrificed 13 days after inoculation
50	8	150	210	4	2,100	Choked 3 diopters	Pneumo. 4th day	Right hemiparesis 3rd day	Sacrificed 4 days after inoculation
127*	4	140	220	5	15	Blurred	Pneumo. 7th day	None	Complete recovery
261*	4	130	140	4	5	No choking	Sterile 7th day	Right hind paw weakness	,, ,,
260	4	150	240	7	15,000	Blurred	Pneumo. in sp. fl. 3rd day	Right fore paw paresis. Stiff neck. Convulsive movements	Died of meningitis 3 days after inoculation
286	4	170	200	8	15	No choking	Pneumo. 10th day	Right hemiplegia, salivation 10th day after inoculation	Died 16 days after inoculation
284	4	150	170	10	10	Normal	Pneumo. 15th day	No neurological signs	Dog died under anesthesia while abscess was being tapped
305*	7	150	160	6	8	,,	Anerobic gram pos. rod 10th day	,, ,, ,,	Abscess drained 10 days after inoculation

*Abscess in these dogs was drained with small rubber tubing. Culture taken at time of drainage.
†Optic disc before inoculation was normal.
Pneumococci disappeared from the abscess in 2 of 19 dogs in which this method was used.

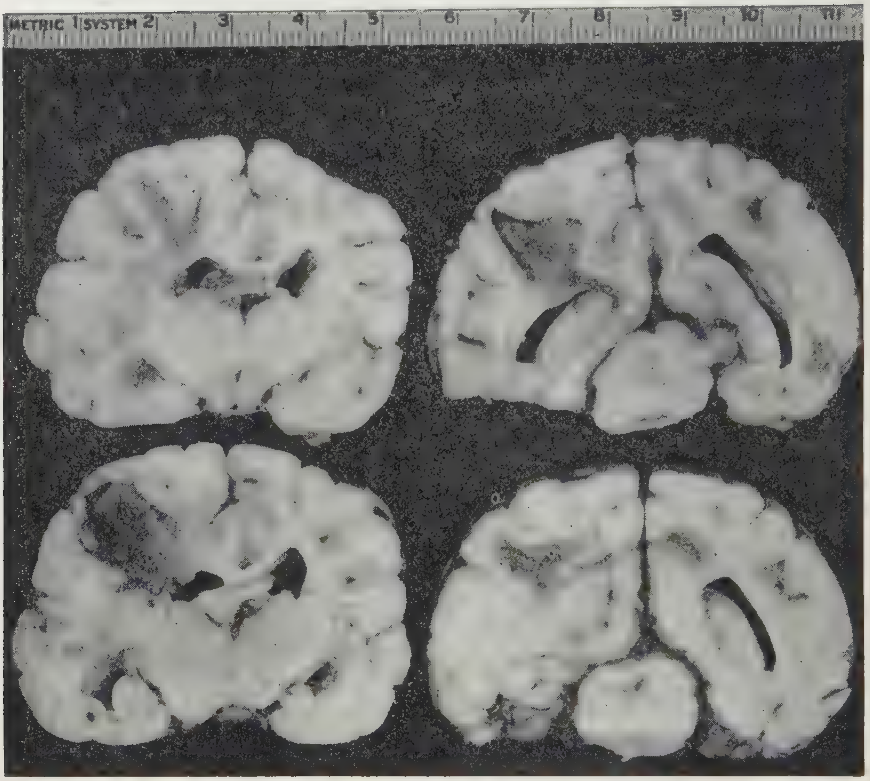

FIG. 1.
Dog No. 124—Coronal sections of the brain.

of these experimental brain abscesses. However, Figs. 1 and 2 are included to show that the experimental abscess conforms in the main to the pathological pictures of brain abscesses from other causes.

Conclusions. 1. A method for the production of experimental brain abscess is reported which will permit the evaluation of chemotherapy and various surgical procedures in the treatment of this lesion. 2. Such abscesses have the gross and microscopic pathological picture of brain abscess from other causes.

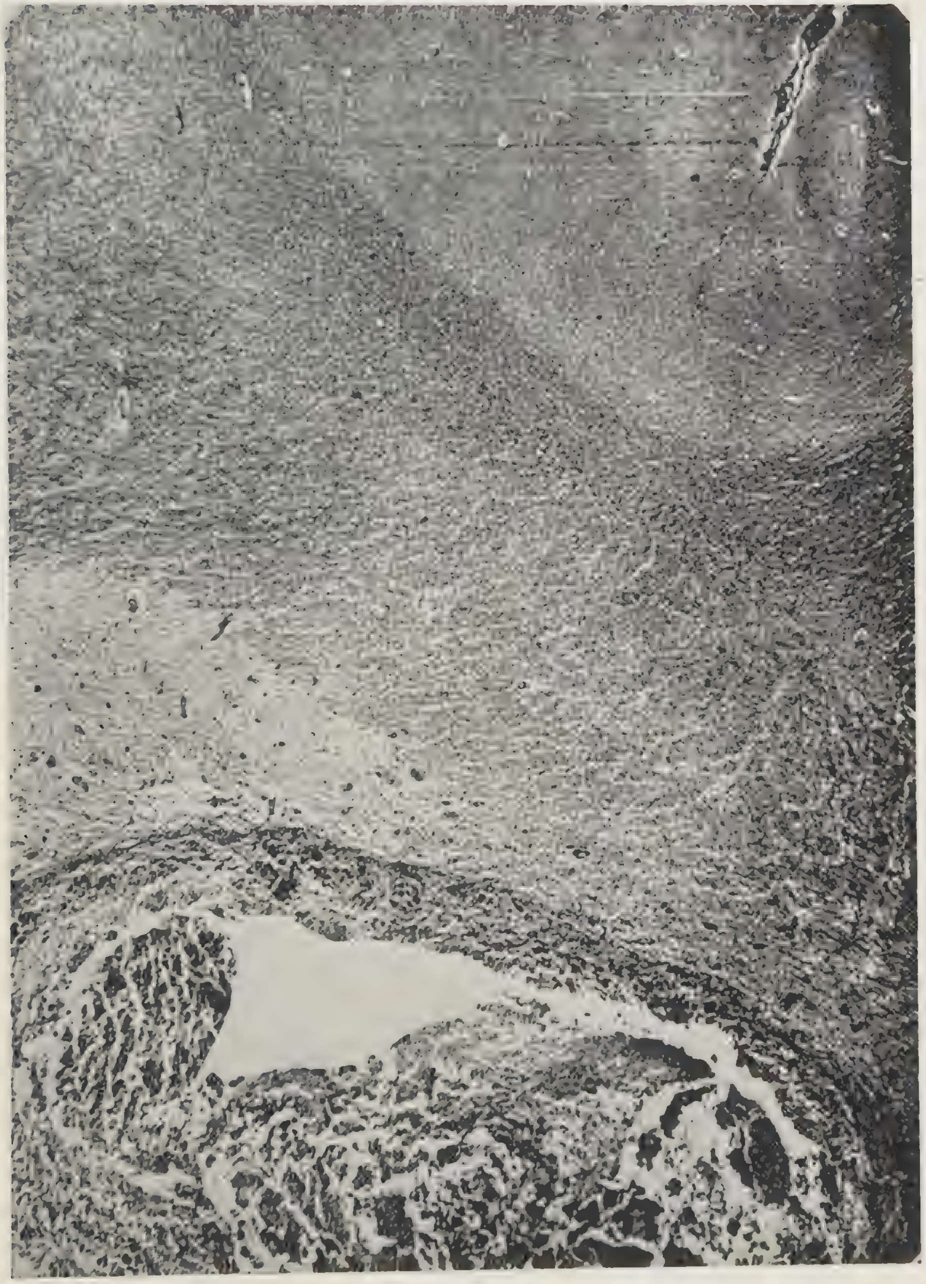

FIG. 2.

Dog No. 124—Photomicrograph showing the necrotic center of the abscess surrounded by an area of infiltration (capsule) and normal brain. × 27.

13076

Physiology of Pure Culture of *Trichomonas vaginalis:* III. Fermentation of Carbohydrates and Related Compounds.

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This study is a continuation of a survey of the physiologic activities of a bacteria-free culture of *Trichomonas vaginalis*.[1-5] Knowledge of the fermentation reactions of this organism is desirable to serve as a basis for the comparison of this species with other pure cultures when they may become available. Moreover, since compounds containing carbohydrates are extensively used in the therapy of vaginal trichomoniasis, the demonstration of any trichomonas-growth-stimulating action of a carbohydrate suggests that it would probably be of limited clinical value.

Experimental. All experiments were performed in duplicate and for those compounds utilized by the protozoa a third test was made.

Utilization of the various substances was determined by comparing the population increase and pH shift in a basic medium with and without the test compounds. Four tubes with the test compound were used in most of the experiments and the results averaged. Population counts were made in hemocytometers (one cubic mm counted per tube). pH measurements were made with glass electrode equipment standardized each time against 2 known buffers.

The basic medium employed and recommended, which gives very poor growth in the absence of utilizable carbohydrates, is prepared as follows:

Two percent proteose peptone No. 3 (Difco), 0.5% NaCl, and 0.1% agar in distilled water adjusted to pH 6, is tubed in 9 cc amounts and autoclaved. After cooling, 0.5 cc of sterile filtered undiluted human serum (adjusted to pH 6 with N/1 HCl) and sufficient autoclaved concentrated sodium thioglycollate solution to give a final concentration of 0.1% are added. The latter is neces-

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[1] Trussell, R. E., *J. Iowa M. Soc.*, 1940, **30**, 66.

[2] Trussell, R. E., and Plass, E. D., *Am. J. Obst. and Gynec.*, 1940, **45**, 883.

[3] Kupferberg, A. B., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 220.

[4] Johnson, G., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 567.

[5] Johnson, G., *Anat. Rec.*, 1940, **78** (suppl.), 179.

TABLE I.
Representative Data Taken from Duplicate or Triplicate Experiments Testing the Fermentation Activities of a Bacteria-free Culture of *Trichomonas vaginalis*.

	A. Experimental cultures in basic medium + test compound				B. Control cultures in basic medium without test compound			Result
Test compound	Uninoculated pH	pH after 3-4 days culture	No. tubes	Avg population per mm^3	Uninoculated pH	pH after 3-4 days culture	Avg population per mm^3	Compound utilized
Monosaccharides								
Arabinose	5.96	6.22	4	1	6.00	6.19	61	0
Rhamnose	5.93	6.10	4	8	6.00	6.16	9	0
Xylose	5.64	5.65	4	3	5.69	5.66	5.5	0
Glucose	6.18	5.22	4	378	6.21	6.17	9	+
Fructose	6.18	6.07	8	104	6.21	6.17	9	±
Galactose	6.12	6.05	3	58	6.21	6.17	9	±
Mannose	6.33	6.27	5	7	6.35	6.40	8	0
Sorbose	6.34	6.38	5	7	6.35	6.40	8	0
Disaccharides								
Lactose	6.02	6.23	4	87	6.00	6.19	61	0
Sucrose	6.00	6.19	4	64	6.00	6.19	61	0
Maltose	6.18	4.69	4	1037	6.21	6.17	9	+
Trehalose	6.00	6.06	4	0.5	6.00	6.08	3	0
Melibiose	6.00	6.10	4	1	6.00	6.16	4	0
Cellobiose	6.00	6.05	3	7	6.00	6.16	4	0
Polysaccharides								
Raffinose	5.70	5.67	4	2	5.69	5.66	5.5	0
Melezitose	6.00	6.20	5	16	6.00	6.08	3	0
Starch (Sol.)	6.18	4.47	3	1260	6.21	6.17	9	+
Inulin	5.79	5.76	4	8	5.69	5.66	5.5	0
Dextrin	6.15	4.66	3	1055	6.21	6.17	9	+
Glycogen	6.15	4.65	3	1268	6.21	6.17	9	+
Pectin	6.13	6.21	5	5	6.35	6.40	8	0
Xylan	6.35	6.36	5	7	6.35	6.40	8	0
Alcohols								
Glycerin	5.73	5.70	4	3	5.69	5.66	5.5	0
Erythitol	5.68	5.70	4	4.6	5.69	5.66	5.5	0
Adonitol	6.00	6.11	5	4	6.00	6.16	4	0
Mannitol	5.66	5.65	3	6	5.69	5.66	5.5	0
Sorbitol	5.99	6.16	5	34	6.00	6.08	3	0
Dulcitol	6.35	6.44	5	8	6.35	6.40	8	0
Glucosides								
Salicin	5.71	5.63	4	17	5.69	5.66	5.5	0
Aesculin	5.70	5.86	5	4	6.00	6.08	3	0
α Methyl Glucoside	6.00	6.15	4	13	6.00	6.16	7	0
Polyhydric Phenol								
Inositol	6.37	6.45	5	8	6.35	6.40	8	0

sary because it was found that "aerobic" fermentation did not occur in its absence.

The 32 compounds listed in Table I were added to the basic medium to a final concentration of 0.5%. The test substances in concentrated solution were sterilized either by filtering or by autoclaving 8 minutes. The basic medium without any additions served as the control.

The inoculums used in test and control tubes were the same in any given set of experiments. Population counts and pH measurements were determined simultaneously. Routine bacteriologic cultures were made from all tubes and no data are included from contaminated specimens.

Under the conditions of these experiments glucose, and its polymers, maltose, soluble starch, glycogen, and dextrin alone were utilized to any considerable extent. Fructose and galactose are in a borderline group as evidenced by a slight stimulation of growth but no appreciable pH shift.

These results differ somewhat from those recorded by Cailleau[6] for *T. foetus* and *T. columbae* and offer one more argument against the identity of *T. vaginalis* and the lower animal trichomonads.

It is interesting clinically that growth of the strain of *T. vaginalis* tested is not stimulated by lactose. This is also true of the common vaginal fungi ("monilia"). Hesseltine[7] contends that lactose should be used in the therapy of vaginitis to prevent the growth stimulation of the fungi which may occur if dextrose is employed. The data here recorded indicate that a similar situation prevails among the trichomonads, although it is obvious that other strains must be studied before such conclusions are warranted.

13077

Susceptibility of Syrian Hamster (*Cricetus auratus*) to Viruses of St. Louis and Japanese B Encephalitis.

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Because of the lack of other susceptible animal hosts, experimental studies on the virus of St. Louis encephalitis have been confined entirely to the use of the mouse and the monkey. Similarly, although the host-range for the Japanese B encephalitis virus appears to be larger,[1] only the mouse and the monkey have proved suitable for laboratory purposes. In view of the desirability, therefore, of having available a fairly large, inexpensive, and readily procurable animal suitable for the study of these viruses, several animal species were examined for their susceptibility to infection. The present report deals with the susceptibility of the Syrian (golden) hamster, *Cricetus auratus.*

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[6] Cailleau, R., *Ann. Inst. Pasteur,* 1937, **59**, 137, 293.

[7] Adair, F. L., and Hesseltine, H. C., *Am. J. Obst. and Gynec.,* 1936, **32**, 1.

[1] Kasahara, S., *et al., Kitasato Arch. Exp. Med.,* 1936, **13**, 248.

Material. The Broun* and Hubbard strains of the St. Louis virus and the Nakayama strain of the Japanese virus were employed. Mouse-passage virus was used to initiate the hamster-passages; virus-infected mouse brains were ground with 10% normal horse-serum broth to make a 10% suspension. After centrifugation at 500 rpm for 2 to 5 minutes, each supernate was injected intracerebrally in 0.1 cc amounts into groups of 2 hamsters. Hamster-brains removed for passage were similarly prepared. All brains were cultured immediately after removal, and only bacteriologically sterile specimens were used.

St. Louis Encephalitis Virus. The Broun strain had been passaged serially through 7, and the Hubbard strain through 9 hamsters. With each strain one hamster-brain from the 5th passage was titrated in mice by the cerebral route. The Broun strain was found to be infectious through a dilution of 10^{-6}, the Hubbard strain through a dilution of 10^{-7} (Table I). A portion of each hamster-brain suspension from this passage was also injected into mice to obtain virus for neutralization-tests. The mice were killed when in convulsions; the brains of 4 were pooled, ground, and made into a 20% suspension in 20% horse-serum broth. The supernate obtained after light centrifugation was used to make serial tenfold dilutions in 10% horse-serum broth. To 0.2 cc of undiluted serum was added 0.2 cc of virus-dilution. The mixtures were incubated for 4 hours at 37°C and each was inoculated in 0.03 cc amounts into groups of 4 Swiss mice. Both strains were neutralized specifically by rabbit antisera to St. Louis virus but not by superimmune lymphocytic-choriomeningitis guinea pig serum.† Sera from normal rabbits and guinea pigs failed to neutralize the virus.

The incubationary period with both strains was 3 to 4 days. Then the fur became ruffled, the animal was apathetic, and the eyes were closed. Salivation was common. The back might become arched, but in the few instances in which this was observed the animal became cachectic and died without showing further symptoms. A fine or a gross body-tremor was frequently discernible

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* This strain was obtained through the courtesy of Dr. L. T. Webster of the Rockefeller Institute.

† The lymphocytic-choriomeningitis antiserum was kindly supplied by Dr. J. E. Smadel of the Hospital of the Rockefeller Institute.

TABLE I.
Titration in Mice of Virus Content of 5th-passage Hamster-brain.

Hamster	Virus and strain	Dilution of hamster-brain				
		10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
H447	St. Louis, Broun	4/4*	4/4	3/4	0/4	0/4
H460	” ” Hubbard	4/4	4/4	4/4	2/4	1/4
H463	Japanese B, Nakayama	4/4	4/4	4/4	4/4	0/4

*The numerator represents the number of mice that died; the denominator, the number of mice inoculated.

and appeared with an ataxia of variable degree. This was followed by paralyses of the limbs, usually the forelegs and spastic in type; flaccidity might occur but was associated chiefly with paralysis of the hind legs. Death supervened within 1 to 2 days after onset of symptoms, although the entire course of the disease might be telescoped into a period of several hours.

The susceptibility of the hamster to the St. Louis virus approximated that of the mouse. Serial tenfold dilutions in 10% horse-serum broth were made from a 10% suspension of mouse-brains infected with the Hubbard strain and inoculated intracerebrally into hamsters (0.1 cc) and mice (0.03 cc). As shown in Table II, the virus was lethal for mice in a dilution of 10^{-7} and for hamsters in a dilution of 10^{-6}. Furthermore, both species appeared to react in a similar way to intranasal, intraäbdominal, or subcutaneous inoculation of large amounts of virus. Thus, of 3 hamsters inoculated intranasally with 0.5 cc of 10% mouse-virus suspension, all died of encephalitis on the 6th day. In another group of 3 animals inoculated intraäbdominally with 0.5 cc of a 1% viral suspension, 2 died of encephalitis and one survived without exhibiting any evidence of infection. None of 3 hamsters inoculated subcutaneously with 0.25 cc of 1% virus showed any signs of infection.

Japanese B Encephalitis Virus. The Nakayama strain was passed serially, brain to brain, through 7 hamsters and its propa-

TABLE II.
Comparison of Infectivity of St. Louis and Japanese B Encephalitic Viruses for Mice and Hamsters.

Virus and strain	Animal inoculated	Dilution of mouse-passage virus				
		10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
St. Louis, Hubbard	Mouse	4/4	4/4	4/4	4/4	0/2*
	Hamster	2/2	2/2	2/2	0/2	
Japanese B, Nakayama	Mouse	4/4	4/4	4/4	3/4	2/4
	Hamster	2/2	2/2	2/2	2/2	

*Two mice died of nonspecific causes within 3 days after inoculation.

gation in this environment is demonstrated by comparing Tables I and II, which show the infectivity for mice of mouse-brain virus (31st mouse-passage) and hamster-brain virus (5th hamster-passage), respectively. Proof was adduced from neutralization-tests that the infectious agent being passed was the Japanese virus and not another latent virus in the host. The agent was neutralized specifically by rabbit antisera to Japanese virus. Normal rabbit or guinea pig sera as well as the sera of guinea pigs immunized against lymphocytic choriomeningitis failed to inactivate the virus.

The incubationary period for the Nakayama strain after cerebral inoculation was 5 to 7 days, somewhat longer than for the strains of St. Louis virus, and the animals exhibited the same clinical picture described above for the St. Louis virus. Like the St. Louis virus, the Japanese virus, by the cerebral route, also possessed about the same degree of infectiousness for the hamster as for the mouse (Table II). The hamster also was susceptible to infection by the nasal route; failure of infection after intraäbdominal or subcutaneous inoculation (Table III), however, was unexpected. While no emphasis is placed on the findings of a single experiment employing only a few animals, it should be pointed out that the latter results are at variance with what occurs when mice are inoculated by the intraäbdominal or subcutaneous routes with the Japanese virus.

Conclusions. The Syrian hamster *(Cricetus auratus)* is highly susceptible to cerebral infection with the viruses of St. Louis and of Japanese B encephalitis and should prove valuable in certain types of investigation where the use of mice or monkeys is not feasible. Both viruses easily propagate in the brain of this host and attain a titer comparable to that achieved in mouse-brain.

TABLE III.
Infectivity for the Hamster of St. Louis and Japanese B Viruses Administered by Various Routes.

	Inoculum		
Virus and strain	Amount	Route	Encephalitis
	cc		
St. Louis, Hubbard*	.5 10^{-1}	intranasal	3/3†
	.5 10^{-2}	inträabdominal	2/3
	.25 10^{-2}	subcutaneous	0/3
Japanese, Nakayama‡	.5 10^{-2}	intranasal	2/3
	.5 10^{-2}	inträabdominal	0/3
	.25 10^{-2}	subcutaneous	0/3

*Lethal dilution for mice = 10^{-7}.

†Numerator denotes number of animals succumbing with encephalitis, denominator the number of animals inoculated.

‡Lethal dilution for mice = 10^{-8}.

13078

Inhibition of Sulfapyridine by the Procaine in Chest Fluids after Procaine Anesthesia.*

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Wood[1] reported the inhibition of bacteriostasis of hemolytic streptococci by sulfanilamide induced by yeast extract. He showed by chemical analysis that the active fraction of the yeast extract was probably identical with p-aminobenzoic acid. He also tested the anti-sulfanilamide action of various compounds related to p-aminobenzoic acid. One of these was an ester derivative, procaine (novocaine). The inhibiting action of procaine was of the same order as p-aminobenzoic acid, though its action was delayed.

Procaine is widely used as a local anesthetic for pleural aspirations and it is possible to demonstrate its presence in the fluids removed, so that a study was undertaken to determine the amount of procaine present, and the inhibitory effect of these amounts on sulfapyridine.

To determine the amount of procaine in pleural fluids, Marshall's test[2] for sulfapyridine was employed, because procaine couples with N (1 naphthyl) ethylene diamine dihydrochloride to give the characteristic red color. With known concentrations of procaine, using a photoelectric colorimeter, a standard curve was drawn.

Pleural fluid[‡] was aspirated from 10 tuberculosis patients under procaine[§] anesthesia using a syringe and needle uncontaminated by procaine; they had received no chemotherapy. The average concentration of procaine in these fluids was 0.0002%. To test the inhibiting effect of these concentrations, sulfonamide inhibitor-free liver media (MacLeod)[3] was employed in all experiments. Tubes containing 5 ml of medium were seeded to contain 1,000 organisms

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[*] This study received additional financial support from the Metropolitan Life Insurance Company, and from Mr. Bernard M. Baruch, Mr. Bernard M. Baruch, Jr., Miss Belle N. Baruch, and Mrs. Robert H. Samstag.

[†] Littauer Fellow in Pneumonia Research.

[1] Wood, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.

[2] Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, **128**, 537.

[‡] Obtained through the courtesy of Dr. Barnet P. Stivelman.

[§] 2% (about 1½ cc).

[3] MacLeod, C. M., *J. Exp. Med.*, 1940, **72**, 217.

[4] Bukantz, S. C., Cooper, A., and Bullowa, J. G. M., accepted for publication in *J. Bact.*

TABLE I.
Inhibitory Effect of Procaine on Bacteriostatic Action of Sulfapyridine Against Pneumococcus III (T3-1).*

Conc. of Sulfapyridine	Concentration of procaine				
	0	.02%	.002%	.0002%	.00002%
%					
0	++++	++++	++++	++++	++++
.003	—	++++	++++	++++	—
.004	—	++++	++++	++++	+
.005	—	++++	++++	++++	—

— no growth.
+ slight growth.
++++ abundant growth.
*Growth observed at 18 hours after incubation at 37°C.

per ml of a standardized strain of Pneumococcus III (T3-1).[4] A series of concentrations of procaine were tested with varying amounts of sulfapyridine. Table I shows that 0.0002% of procaine, which is the average amount found in plural fluids of patients thus anesthetized, is sufficient to inhibit the action of 0.003, 0.004, and 0.005% of sulfapyridine.

It is possible that after procaine anesthesia sufficient procaine may be present in the remaining chest fluid to at least temporarily inhibit the action of sulfapyridine and permit bacterial growth. The effect of urethane on sulfapyridine was studied because it does not couple in the Marshall test. It was found that in concentrations as high as 0.05% urethane did not inhibit sulfapyridine.

Summary. 1. The average concentration of procaine in pleural fluids removed with this anesthetic was 0.0002%. 2. Sulfapyridine in concentrations as high as 0.005% was inhibited by procaine *in vitro.* 3. Concentrations of urethane as high as 0.05% do not inhibit the action of sulfapyridine *in vitro.*

13079

Influence of Prolonged Electrolyte Deprivation and Final Restoration on Fluid Intake, Balance and Distribution.

J. W. REMINGTON, W. M. PARKINS AND H. W. HAYS. (Introduced by W. W. Swingle.)

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Gilman[1] found that the intravenous injection of an hypertonic salt solution, which increased the volume of the plasma and interstitial fluid at the expense of the intracellular compartment, markedly increased the water intake of the dog. Conversely, confirming Darrow and Yannet,[2] when the extracellular compartment was dehydrated and the intracellular volume increased after the intraperitoneal injection of an isotonic glucose solution, the water intake was reduced.

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[1] Gilman, A., *Am. J. Physiol.*, 1937, **120**, 323.

In the present study, the extracellular electrolytes of 7 dogs were partially depleted by intraperitoneal glucose injections and then maintained at low levels for a period of 5 to 15 days through the use of a salt-free diet, to make observations on the following questions: (1) Whether the fluid shifted into the intracellular compartment to restore osmotic equilibrium between it and the electrolyte depleted extracellular compartment would be spontaneously returned. (2) Whether the reduced voluntary water intake would be maintained, and could be correlated with intracellular volume. (3) Whether the injection of NaCl would restore normal fluid distribution and water intake.

Methods. An intraperitoneal injection of 100 cc per kg body weight of a 5.5% glucose solution was given each animal, paracentesis being performed at the end of 5 hours. The animals were placed in metabolism cages, and water intakes, urine volumes and urine electrolyte analyses recorded daily. Serum electrolyte analyses were made at selected intervals. Food was withheld for the first few days. Then salt-free meat[3] was given in measured amounts. In constructing fluid and electrolyte balances, it was assumed that the initial total body water was 65% and the extracellular volume 27% of the body weight. Experimental extracellular volumes were derived from serum sodium and chloride concentrations[4] and from hematocrit and hemoglobin readings. It was found that for the first 7-10 days of the experiment all 4 methods of derivation gave comparable extracellular volumes, but that later hematocrit and hemoglobin readings would indicate an abnormal dilution. The discrepancy might be due to a progressive anemia of dietary origin.

Corrections for metabolic water gain and extra-renal loss were made by assumed constants based on previous experiments. Intracellular volumes were taken to be the difference between total body water and the average extracellular volume as derived from serum sodium and chloride concentrations.

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[2] Darrow, D. C., and Yannet, H., *J. Clin. Invest.*, 1935, **14**, 266.

[3] Swingle, W. W., Parkins, W. M., Taylor, A. R., and Hays, H. W., *Am. J. Physiol.*, 1936, **116**, 438.

[4] Harrison, H. E., Darrow, D. C., and Yannet, H., *J. Biol. Chem.*, 1936, **113**, 515.

TABLE I.
Fluid Intake, Balance, and Distribution in Dogs Subjected to Prolonged Electrolyte Deprivation.

Date	Body wt, kg	Serum Na, m-eq/l	Serum Cl, m-eq/l	Serum K, m-eq/l	Hematocrit, %	Avg water drunk, cc	Avg urine volume, cc	Avg water balance, cc	Avg urine Cl, m-eq	Avg urine Na, m-eq	Avg urine K, m-eq	Avg extracellular volume from serum Na and Cl, l	Intracellular volume, l
						Dog 1.							
10/21[1]	11.0	146.6	113.8	4.6	53.2							2.97	4.18
10/21[2]		129.3	92.8	5.1	68.7	0	0					2.29	4.44
10/22	10.5	129.8	93.0	5.5	61.2	0	0	—422				2.28	4.44
10/25[3]	9.5	129.3	95.8	6.3	62.9	637	737	—184	9.5	0.6	28.1	2.11	3.84
11/1	9.3	133.4	92.8	3.7	57.0	1813	2005	— 16	8.3	1.4	9.5	1.90	3.83
11/2[4]						1485	1320	+465	63.2	29.2	10.7		
11/3	10.1	148.1	111.8	4.0	45.5	290	230	+360	11.4	6.5	1.5	2.75	4.18
						Dog 2.							
9/21[5]	17.2	146.7	112.8	4.7	50.6							4.64	6.54
9/21[6]		127.1	86.8	3.8	61.8	0	0					4.20	6.98
9/22	16.1	136.9	96.4	4.0	55.6	0	490	— 634	2.5	0.6	11.5	3.86	6.69
9/24[3]	15.2	138.8	99.4	6.2	53.6	460	570	— 254	3.0	0.5	12.5	3.70	6.34
9/27	15.7	137.2	97.8	5.2	53.7	970	1227	— 19	3.6	1.1	19.1	3.69	6.28
9/30		138.8	100.0	5.3	51.0	853	1197	— 106	4.3	0.8	12.5	3.58	6.30
10/7	15.4	140.7	103.8	5.3	50.0	620	850	+ 8	4.0	0.3	10.9	3.59	6.33
10/8[7]						1175	291	+1122	3.7	18.4	8.8		
10/9	16.5	149.8	117.8	4.7	41.2	40	280	— 2	20.1	13.0	10.1	4.47	6.40

[1] Injected 1100 cc glucose solution.
[2] Drained 1440 cc peritoneal fluid containing 147.3 m-eq Na, 121.0 m-eq Cl.
[3] Started salt-free diet, 500 g daily.
[4] Given 9 g NaCl, plus salted food.
[5] Injected 1720 cc glucose solution.
[6] Drained 1720 cc peritoneal fluid containing 177.3 m-eq Na, 138.8 m-eq Cl.
[7] Given 10 g NaCl plus salted food.

Results. The balance data on 2 representative dogs are given in Table I. Complete equilibrium between all parts of the extracellular fluid had often not been reached at the time of paracentesis, as evidenced by a later rise in serum electrolyte concentrations (*e. g.,* Dog 2). Constant levels had been attained by the 24th hour. Throughout the period in which NaCl was restricted these concentrations showed either no change or a slight progressive rise. In no case was there a dilution of extracellular electrolyte, as would be expected if fluid were being returned from the intracellular compartment. These results agree with those reported by Mellors, Muntwyler and Mautz,[5] based on similar experiments.

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[5] Mellors, R. C., Muntwyler, E., and Mautz, F. R., *Proc. Am. Physiol. Soc.,* Chicago, 1941.

The initial response to the electrolyte deprivation was a complete absence of water intake and a persistent negative water balance. With constant extracellular volumes, this body water loss must have been contributed by the intracellular compartment. Usually by the 3rd day this compartment had lost a volume equal to that originally gained upon the intraperitoneal glucose injection, and had reached a stable minimal value. Since this initial period was one of starvation, it was to be expected that this value should be lower than that typical of the preëxperimental period.

Coincident with the attainment of this stable intracellular volume was the first voluntary taking of water, which either greatly minimized the negative water balance or restored equilibrium. Water intakes now rose steadily until they reached levels of polydipsia. Urine volumes rose accordingly, however, so that in no case was there a positive water balance established, despite the fact that the extracellular compartment was still dehydrated. Intracellular volumes were not restored to the preëxperimental level, although food was taken during this period.

When NaCl was given intravenously in amounts roughly equal to that originally removed in the peritoneal fluid, extracellular electrolyte concentrations were immediately restored to normal. The water intake on the first day of salt restoration was always high, and often twice that of previous days. A marked positive water balance was established, due in part to this increased intake and even more to a marked reduction in urine volume. The high water intake was not maintained on subsequent days, although the water balance was usually still positive. Extracellular volumes were not immediately restored to the preëxperimental level. This fall in intake might be coincident with a secondary rise in intracellular volume which occurred, with restoration to the preëxperimental levels.

Summary. Dogs partially depleted of extracellular electrolytes by intraperitoneal glucose injections and then maintained on a salt-free diet showed absence of fluid intake and a negative water balance while the intracellular volumes were above normal. When these volumes had been reduced to stable minimal levels, water was taken by mouth and the negative water balance corrected. Although the intakes now rose to polydipsic levels, a positive balance was not established despite the fact that the extracellular volumes were still reduced. Intracellular volumes were not restored to the preëxperimental level. When NaCl was given, extracellular electrolytes and volumes were restored toward normal. The initial response was an increased intake which, however, was not sustained. The intracellular compartment, originally decreased, showed a delayed rehydration to normal levels. Hence the voluntary water intake showed more positive correlation with changes in intracellular volume than with extracellular volume change. Fluid balance was affected both by intracellular hydration and electrolyte levels in the extracellular fluid.

13080

Widened Reactivity of Antibody Produced by Prolonged Immunization.

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A number of workers have observed that antibody becomes more cross-reactive as the course of immunization is prolonged. The first observations were apparently those of Magnus[1] on antigenic plant-extracts. Many similar results have been obtained with antigenic cells or crude mixtures of antigens; the first evidence that reasonably pure proteins can produce the same effect was that of Wells and Osborne.[2] We used 5-times recrystallized ovalbumins from hen and duck and offered additional proof[3] that the antibodies to a single "pure" antigen may develop a broadened reactivity as immunization proceeds. The antisera from later bleedings *may* show a broader equivalence-zone,[4] and the antibody, in the zone of antigen-excess, can combine with a larger quantity of antigen.[4, 5]

Three possible explanations of these effects have been considered; conceivably all of them might apply in some instances.

1. The antibody initially formed is directed toward a dominant determinant-group of the antigen, and, progressively, additional antibodies are formed for separate, minor determinants.[6–9]

2. The later antibodies differ from the earlier by the presence on

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[1] Magnus, *Ber. deut. Bot. Gesellsch.*, 1908, **26**a, 532 (cited in[2]).

[2] Wells, H. G., and Osborne, T. B., *J. Inf. Dis.*, 1913, **12**, 341; 1916, **19**, 183.

[3] Hooker, S. B., and Boyd, W. C., *J. Immunol.*, 1934, **26**, 469; 1936, **30**, 41.

[4] Heidelberger, M., and Kendall, F. C., *J. Exp. Med.*, 1935, **62**, 697.

[5] Malkiel, S., and Boyd, W. C., *J. Exp. Med.*, 1937, **66**, 383.

[6] Hooker, S. B., and Boyd, W. C., *J. Immunol.*, 1933, **25**, 61.

[7] Heidelberger, M., and Kendall, F. C., *J. Exp. Med.*, 1934, **59**, 519.

[8] Landsteiner, K., and van der Scheer, J., *J. Exp. Med.*, 1938, **67**, 709.

[9] Pauling, L., *J. Am. Chem. Soc.*, 1940, **62**, 2643.

their molecules of additional discrete reactive groups that may differ qualitatively, the later antibodies thus being more multivalent.[4, 10, 11]

3. The later antisera contain antibodies whose combining groups, although not necessarily more than one per molecule, possess a wider affinity, which permits combination with a larger number of related chemical groups, perhaps because the reactive patch on the molecule of antibody is larger and its pattern is more complex. Combination with related heterologous haptens or antigens might be effected by limited portions of the chemically active groups constituting the pattern of the antideterminant, the whole structure being brought into play only in the presence of the complete (homologous) determinant.

The third possibility is substantially an extension of the explanation offered by Landsteiner[12] to account for the multiplicity of antibodies formed to one determinant-group (metanilic acid); that is, the antibodies " . . . . vary to some extent around a main pattern." Such structural similarities also seem adequate to explain the cross-reactivity of antisera to various pure ovalbumins.[13] The variation among antibodies is strikingly shown by absorption with heterologous antigens and we think that the behavior of such absorbed sera, when further treated with heterologous hapten[14] or antigen,[13] offers support for the above-mentioned concept that cross-reactions may often depend on the activity of only a portion of the pattern of chemical groups (perhaps not necessarily even adjacent groups) composing the reactive patch on the molecule of antibody.

We may point out that the antibodies to hen-ovalbumin[13] (H) could be separated by absorption with turkey-ovalbumin (T) into at least two kinds: those able to precipitate T and those which could not. The latter were nevertheless prevented from precipitating H, by the addition of a 16- to 32-fold relative excess of T. The indicated conclusion is that all of the anti-hen precipitins had the power of reacting with T, but that those which could precipitate it—and according to our own experience these would be produced later in the course of immunization—had a still coarser specificity, enabling them to combine more firmly and produce an insoluble compound.

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[10] Pappenheimer, A. M., Jr., *J. Exp. Med.*, 1940, **71**, 263.

[11] Heidelberger, M., Treffers, H. P., and Mayer, M., *J. Exp. Med.*, 1940, **71**, 271.

[12] Landsteiner, K., *The Specificity of Serological Reactions*, C. C. Thomas, Baltimore, 1936, p. 143.

[13] Landsteiner, K., and van der Scheer, J., *J. Exp. Med.*, 1940, **71**, 445.

[14] Landsteiner, K., and van der Scheer, J., *J. Exp. Med.*, 1936, **63**, 325; 1931, **54**, 295.

During a study[15] of the cross-reactivity of antisera to *p*-amino-benzoic-acid-azohemocyanin *(Limulus)* we tested the inhibitive capacity of a number of uncoupled compounds more or less related to the prosthetic group of this compound antigen, and observed that some antisera from later bleedings contained antibody capable of being inhibited by cyclohexane carboxylic acid, whereas this compound did not inhibit precipitation by antibody from earlier bleedings of the same animals. We were skeptical of the accuracy of the first observation but have repeatedly confirmed it.

Method. After preliminary titrations to determine the optimal amounts of immune serum and test-antigen a quantity of the latter, *p*-amino-benzoic-acid-azocasein, sufficient to give a concentration of 2 γ N/ml was mixed with 5% gelatin in salt solution.[16] About 0.2 ml amounts of this mixture were distributed in small tubes (5 mm bore) and chilled. Equal parts of diluted serum and the serially diluted compound being tested for inhibitive property were mixed and overlayered on the preparation of antigen, and the tubes were kept at 37° for 5 hours. Reactions at the sharp interface thus ensured were recorded at hourly intervals. Inhibition by very small amounts of some compounds was complete even after refrigeration overnight. The compounds were adjusted to pH 7.4 or as near thereto as solubility permitted. They did not inhibit unrelated sera.

An example of the inhibitive behavior of cyclohexane carboxylic acid (CCA) is given in Table I. Similar results were obtained with sera from another rabbit but not from a third. It will be noted that the disparity between the effectively inhibitive amounts of the different haptens is much greater in the case of the 2nd than of the 3rd or 5th bleeding. The fact that the early antibody is inhibited by the homologous hapten and not by CCA whereas later antibody

TABLE I.
Precipitation of Casein-azo-*p*-aminobenzoic Acid with Antihemocyanin-azo-*p*-aminobenzoic Acid Inhibited by Simple Haptens.

	p amino benzoic acid			Benzoic acid			Cyclohexane carboxylic acid		
	Micromols required to inhibit completely for								
Serum*	1	2	3	1	2	3	1	2	3 hr
562_2 1:10	.174	.174	.260	.392	.588	.588	not by 2.00		
562_3 1:6	.077	.116	.174	.077	.174	.260	.116	.392	.588
562_5 1:4	.077	.174	.260	†	†	†	.116	.174	.260

*Subscripts indicate number of bleeding of rabbit 562.
†Not tested.

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[15] Hooker, S. B., and Boyd, W. C., *Arch. Path.*, 1939, **28**, 754.

[16] Hanks, J. H., *J. Immunol.*, 1935, **28**, 95.

is temporarily, but completely, inhibited by CCA indicates that the early kind of antibody has virtually disappeared from the serum and has been replaced by antibody capable of combining with the heterologous hapten. In this instance an interval of 7 weeks had elapsed between the 2d and 3d bleedings—an interval probably far longer than necessary for the elimination of early antibody and for a fresh antibody-response to the injections of antigen given during this intervening period.

The development of this broadened reactivity seems to be accounted for best by the third explanation offered above. The mechanism suggested in the first, which is surely sometimes operative, is less applicable in this case which concerns a relatively simple hapten; the second possibility, involving an increased valence (in the sense of discrete combining patterns) of the antibody-molecule, can hardly apply at all.

Structurally, the haptens here considered are grossly very similar and the influence of finer configurational differences among them must be invoked to explain the results obtained. One may consider the influence of the presence of double bonds[17] and an (altered) NH_2 group which might dominate the production of early antibody; different angles between the carbon atoms in the two kinds of ring, or different planarities of the rings as wholes. We are informed that the oxygen atoms in benzoic acid would tend to form stronger hydrogen bonds with proton-donating groups than would those in CCA; if hydrogen bonds are concerned in the union of antigen with antibody[18] then their influence on specificity would appear to be greater in the case of the early antibody, which was inhibited by benzoic acid but not by CCA. Whether any of these possible influences is operative cannot be decided at present; the nature of the altered mechanism of antibody-formation is entirely obscure.

Summary. Specific precipitation by antibody produced early in the course of immunization with *p*-amino-benzoic-acid-azohemocyanin was not inhibited by a related heterologous hapten, cyclohexane carboxylic acid. As immunization continued the antibodies were replaced by one that was strongly inhibited by this hapten. Some possible reasons for this broadened reactivity are discussed.

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[17] Erlenmeyer, H., and Berger, E., *Arch. exp. Path. u. Pharm.*, 1934, **177**, 116.

[18] Pauling, L., *The Nature of the Chemical Bond*, Cornell Univ. Press, Ithaca, 1939, p. 411.